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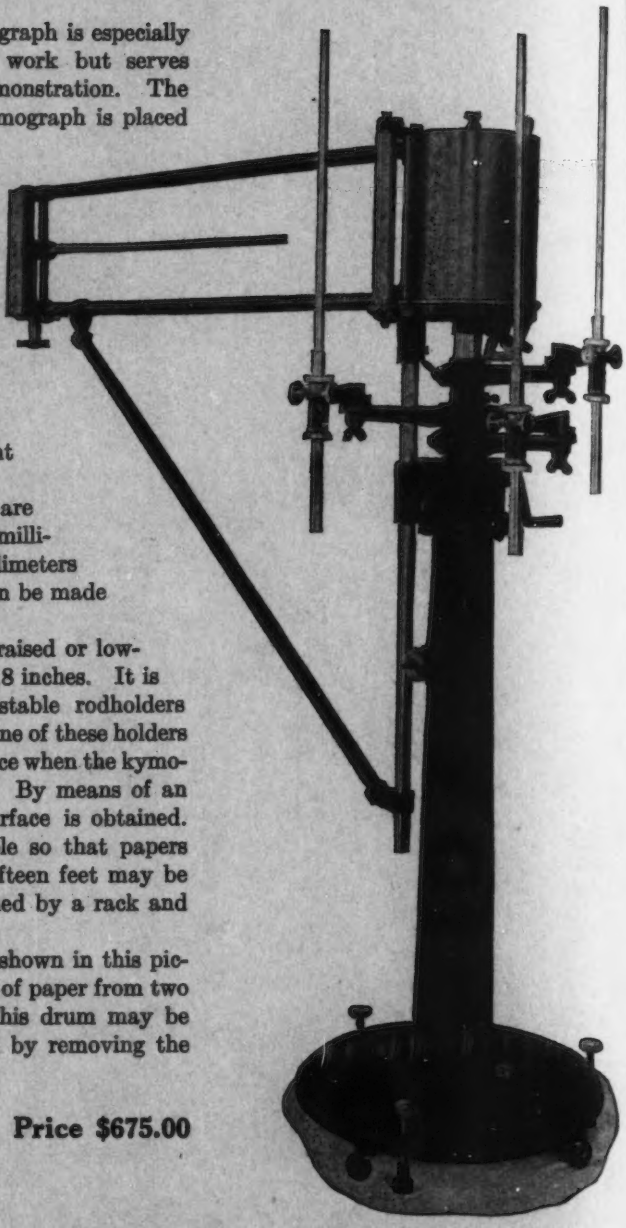

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THE EFFECT OF LUMBAR SYMPATHECTOMY ON THE FLOW OF BLOOD IN THE FEMORAL ARTERY OF THE DOG

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In recent years sympathectomy has been employed in an attempt to relieve certain pathologic conditions such as Raynaud's disease and other diseases in which the peripheral circulation of the extremities is inadequate. As observed clinically, definite improvement has followed such operations in some of these conditions. The improvement, in all probability, is due to an increased flow of blood to the extremities, owing to the release of the vessels from vasoconstrictor influence (1) (2). Indirect evidence of increased flow of blood to the extremities following lumbar sympathetic ganglionectomy in the dog has been reported by Sheard, Rynearson and Craig (5). A disease such as Raynaud's cannot be duplicated in animals, yet the effect of sympathectomy on the flow of blood to the extremities can be definitely determined. Since information of this nature might prove of value, and since quantitative measurements of flow of blood before and after sympathectomy on animals have not been reported, a series of experiments was performed in order to ascertain the quantitative alterations in flow in the femoral artery following lumbar sympathectomy in the dog. Herrick and Baldes have given a detailed description of the thermostromuhr method of Rein and its modification for measuring flow of blood.

In brief, the thermostromuhr method consists of heating the blood at a given place as it flows through the vessels, and of detecting the rise in temperature resulting from this heating. The blood is heated by applying electrodes which are connected to a high frequency circuit. The rise in temperature is measured indirectly by means of a differential thermocouple (the thermojunctions being placed equidistant above and below the place of heating) connected to a Zeiss loop galvanometer. This rise in temperature is a function of the flow of blood. Figure 1 illustrates the compendious unit called the diathermy thermo-element, containing heating

electrodes and thermojunctions, which is applied to the blood vessel in situ. The blood vessel is carefully isolated and freed from its connective tissues only so far as is necessary for insertion into the groove of the diathermy thermo-element. The wound is closed tightly and sufficient time is allowed for establishing heat equilibrium before any observations are made.

The initial experiments were acute and were performed under ether anesthesia. After the flow in each femoral artery was established, the entire chain of ganglia from the level of the second lumbar vertebra posteriorly to the end of the chain was removed (occasionally as was determined at necropsy the second lumbar ganglion was missed). Immediately following this operation the flow in each femoral artery was again measured. The results of these experiments were inconclusive since in some cases a

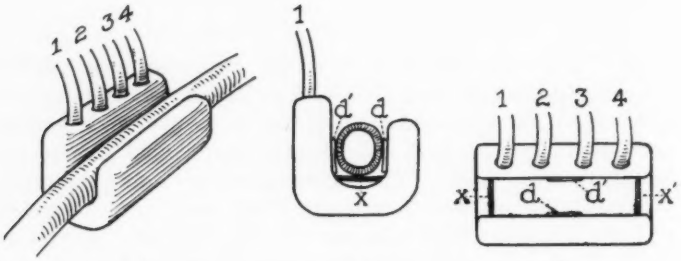

Fig. 1. Diathermy thermo-element. *A* shows its application on a blood vessel; *B* shows a cross section of the blood vessel as it fits against the electrodes (platinum plates) d and d' and on a thermojunction x. *C* shows the relative positions of plates and thermojunctions. x and x' are connected to the Zeiss loop galvanometer by leads *1* and *4*. d and d' are connected to leads *2* and *3*.

moderate increase in flow followed the sympathectomy, whereas in others significant changes were not observed. In certain experiments measurements were taken simultaneously with the removal of succesive ganglia in the hope that this procedure would reveal the relative influence of each ganglion on the flow of blood to the limb. The results of these experiments were also inconclusive. It was suspected that ether anesthesia was responsible for the variable results. It was considered logical that the depth of anesthesia should materially influence the flow of blood to the limb. It is evident that in cases in which the anesthetic has produced complete relaxation of the vessels, sympathectomy should be incapable of causing much further dilatation. Support is given to these views by data presented later in this report.

Since a general anesthetic was considered inadvisable, subsequent ex-

periments were done under local anesthesia, the lumbar sympathectomy having been previously performed under ether anesthesia and sterile technic. The dogs were allowed to recover completely from the operation which usually required about two weeks. During this period the dogs were trained daily to lie quietly on the table and they thus became accustomed to the characteristic noise of the diathermy machine and also to the workers. At the end of the training period a comparative study of the flow in the femoral arteries was made, the artery on the intact side serving as the control. After the flow in one femoral artery was determined the same diathermy thermo-element was transferred to the other femoral artery and its flow established. In order to eliminate the personal equation involved in the observations, the sympathectomized side was unknown to the observer.

In a series of dogs of approximately the same weight the flow of blood was measured on the sympathectomized and intact sides under local anesthesia. The definite results of these experiments are in striking contrast to the inconclusive results obtained under general anesthesia. In this series the sympathectomized side showed a much greater flow of blood than the intact side in every instance; except in one case the flow in the former was twice as great (table 1).

As a result of the experiments described our interest was naturally aroused as to the difference in the flow in the right and left femoral arteries of normal dogs. For this study a series of apparently normal dogs was chosen and a comparison of the flow in the right and left femoral arteries was made first under local and then under ether anesthesia. The results of these experiments were surprisingly constant since the flow in the two arteries was found to be the same within the range of experimental error whether the experiment was done under local or general anesthesia. As was suspected after our early experiments, the flow under ether anesthesia showed a marked increase over that under local anesthesia in every instance (table 2).

The final experiments were devoted to a study of the relative influence of sympathectomy and general anesthesia on the flow in the femoral artery. As was indicated previously, changes in flow of blood were not detected after sympathectomy when a general anesthetic such as ether was used. It seemed probable that this might be due to the release of the vessels of the limb from vasoconstrictor influence. It was supposed that a general anesthetic brought about such complete relaxation of the vessels that any further dilatation which might be caused by sympathectomy could not be detected. In order to shed some light on this problem, the following experiments were done.

Two dogs were trained and a comparative study of the flow in the femoral arteries was made under local anesthesia. Immediately following

these observations, the dog was given a general anesthetic (ether) and the flow in each femoral artery again was determined. When the dog had recovered from the effects of this experiment a lumbar sympathectomy was performed as in previous instances. Two weeks after the operation, the flow in the femoral arteries was again studied under both local and general anesthesia. The results of these experiments demonstrate rather conclusively that the vasodilatation produced by deep general anesthesia is apparently as great as that caused by sympathectomy (table 3).

TABLE 1

Comparative blood flow in the femoral arteries of dogs following sympathectomy on the left side

DOG	WEIGHT	ARTERIAL FLOW PER MINUTE	
		Right femoral	Left femoral
	kgm.	*cc.*	*cc.*
1	13.6	215	377
14 days later	13.6	152	320
2	13.7	54	105
3	13.2	125	294
14 days later	13.2	131	293
4	11.2	165	383

TABLE 2

Comparative blood flow in the femoral arteries of normal dogs under local and general anesthesia

DOG	WEIGHT	ANESTHESIA	FLOW PER MINUTE	
			Right femoral	Left femoral
	kgm.		*cc.*	*cc.*
1	17.4	Local	89	82
		General	132	132
2	17.8	Local	80	77
		General	236	254
3	19.2	Local	64	63
		General	107	117

TABLE 3

Flow in femoral arteries in cubic centimeters per minute

	NORMAL		AFTER LEFT SYMPATHECTOMY	
	Local anesthesia	General anesthesia	Local anesthesia	General anesthesia
Right femoral	80	236	84	263
Left femoral	77	254	201	260

SUMMARY AND CONCLUSIONS

A series of studies has been made on the flow in the femoral artery of the dog. A comparison has been made of the minute volume flow in the right and left femoral arteries using both local and general anesthesia. After unilateral sympathectomy the flow was again measured. The results show that the flow is the same in both femoral arteries of the normal dog. Following unilateral sympathectomy the flow in the femoral artery on the sympathectomized side is about twice as great as that on the intact side. When

ether anesthesia is used in place of local infiltration there is not an outstanding difference between the intact and sympathectomized sides. It may be concluded that surgical ether anesthesia is almost as effective in producing vasodilatation as sympathectomy.

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THE MALE HORMONE

V. The Effect of the Male Hormone and the Anterior Pituitary

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Very soon after our initial experiments, in which we showed the presence of the male hormone in the male urine (1; see also 2–6), an elaborate series of tests on the effect of the hormone on old rats was begun. For this purpose, male rats from the Wistar Institute, as near the maximum age (plus or minus 3 years) were used. Each male rat was kept with two young female rats for a sufficiently long time to make certain that the male rat was sterile. The old rats were next divided into controls and experimental animals, and the latter were daily injected with the male hormone over a period of as many weeks as they continued to live. In no case could it be shown that the old, impotent rats could be made potent by male hormone injections.

In the meantime, the experiments of Smith and Engle, Ascheim and Zondek and others (7, 8) have made it evident that the anterior pituitary is responsible for stimulating the sex process in the female and in the male. As a preliminary to certain work we have in mind, the effect of the injection of the anterior pituitary hormone and the male hormone upon the seminal vesicles of the young rat has been studied.

Experimental. For purposes of comparison, we divided our rats, around a month old, into four groups: 1, controls; 2, injected with the male hormone; 3, injected with the anterior pituitary; 4, injected both with the male hormone and the anterior pituitary combined. In the first series of experiments, the rats were injected daily for six days, then killed and their seminal vesicles examined.[1] The very striking results obtained caused us to repeat these experiments, but this time *the animals were killed at the end of four days.* During the experimental period, each animal received a daily injection of 0.5 cc. of an extract of male hormone (the strength of which we shall presently discuss), and a twice daily injection of 0.3 cc. of the urine of pregnant women. The present uncertainty with regard to

[1] We are very much indebted to Dr. Alfred Plaut, of the Beth Israel Hospital, New York, for the histological examinations.

the potency of commercial extracts of anterior pituitary made us choose, for the time being, the urine of pregnant women itself.

We have described, in previous publications, our methods for preparing potent extracts of the male hormone. A brief description of the method employed in preparing the male hormone extract used in these experiments may here be given. The urine is strongly acidified and extracted with chloroform, under reflux, for eight hours. The chloroform portion is drawn off, the chloroform distilled, and the residue heated under reflux, for two hours, with 20 per cent sodium hydroxide. The product is repeatedly extracted with ether, the ether extract is evaporated, and the residue taken up in oil.[2] One cubic centimeter of the final product is equivalent to 10 cock units. The "cock unit" is defined as that amount of extract which will cause an increase in the size of comb and wattles of 20 per cent over the initial value in the course of 10 days. For each test five capons are employed. One "cock unit" represents the equivalent of about 125 cc. of urine.

From a number of experiments, all confirmative in character, we select the following as typical examples (compare with the photographs):

A. Control. Rat weighed 40 grams. At end of the experiment, the seminal vesicles were thin and glassy, 3.5 mm. long and 1.1 mm. wide. The ducts were thin.

A′. Control. Rat weighted 34 grams. The picture similar to A. The seminal vesicles were 4 mm. long and 1.5 mm. wide. (This one is not shown in the figure.)

B. Injected with male hormone. Rat weighed 35 grams. The seminal vesicles were not so thin and glassy as those of A and A′; they were 6 mm. long to 2 mm. wide. The ducts were thin and grayish.

C. Injected with male hormone. Rat weighed 30 grams. The vesicles were of the same appearance as B, and they were 5 mm. long and 1.5 mm. wide.

D. Injected with anterior pituitary. Rat weighed 40 grams. The seminal vesicles were grey and thin, 7.5 mm. long up to 3.5 mm. wide. They were conical in shape. The average width was less than 3.5 mm.

E. Injected with anterior pituitary. Rat weighed 33 grams. The seminal vesicles were 8 mm. long and 2.5 mm. wide.

F. Injected with the male hormone and the anterior pituitary. Rat weighed 41 grams. The seminal vesicles looked quite different; they were much thicker, and not glassy but opaque, having the ordinary yellowish pink color of organs. The seminal vesicles were 8 mm. long and 3 mm. wide, and very thick and folded. The ducts were considerably thicker than in the other groups (A–E).

[2] For technical assistance in these preparations we wish to thank Mr. Barnet Naiman, of the College of the City of New York.

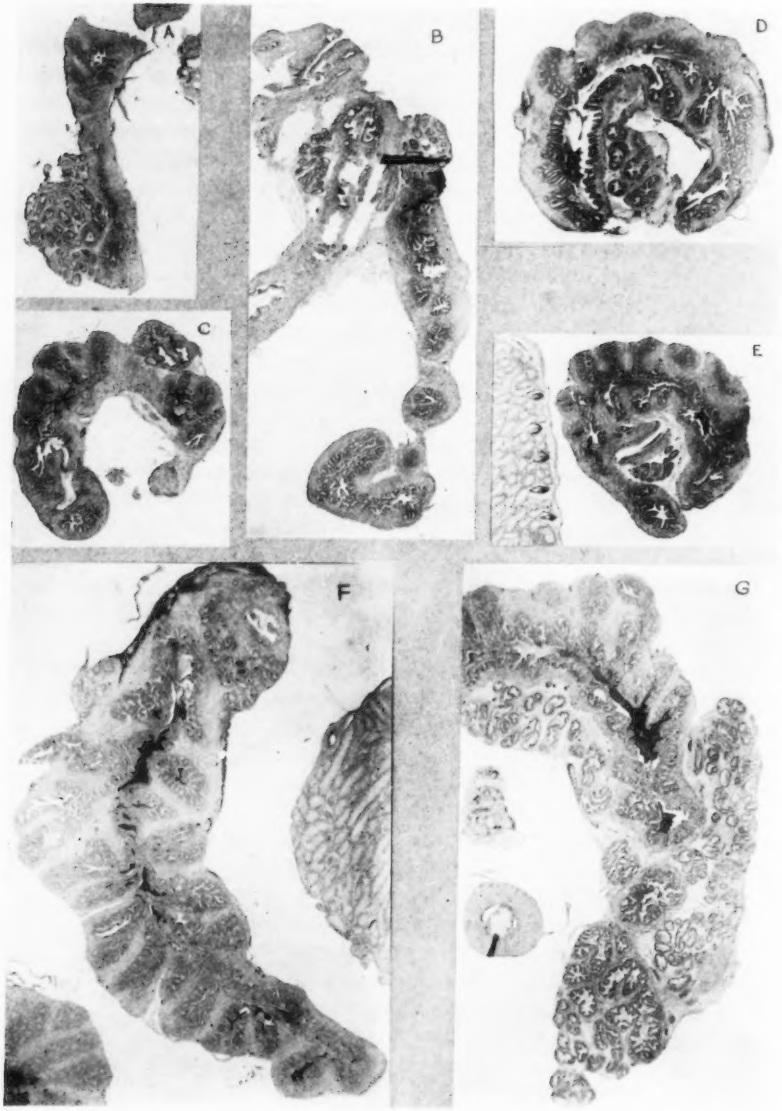

Fig. 1

G. Injected with the male hormone and the anterior pituitary. Rat weighed 35 grams. The seminal vesicles were 8.1 mm. long and 3.1 mm. wide, being very thick as in F.

DISCUSSION. The controls showed not only extremely small seminal vesicles (S. V.) but there was very little glandular development, and hardly any secretion. The S. V. of the animals injected with the male hormone were somewhat larger and they contained more secretion. As is well known, and as Moore and others (9, 10, 11) have shown, by injecting castrated rats with the male hormone, one can prevent the development of castration changes in the S. V. In fact, Moore and his co-workers have used this and a number of other methods for assaying the male hormone (12). Somewhat larger were the S.V. of the animals injected with the urine of pregnant women, and they showed a little more secretion. In this connection it may be of interest to note that Smith and Engel (7) found that the genital system of immature male rats is less responsive to anterior pituitary transplants than female rats, and that where mature male rats are used, no effect on the genital system is observed. However, Collip (13) found that by using an anterior-pituitary-like hormone obtained from human placenta, and injecting it into rats, 18 to 24 days old, for a period of 10 to 50 days, the S. V. showed appreciable increases in size. Somewhat similar results were obtained with adult male rats, $2\frac{1}{2}$ to 8 months old.

Where the rats received both the male hormone and the urine of pregnant women, the pictures of the seminal vesicles were outstanding: the organs were large, there were many ramifications of the glandular structure, and there was much secretion.

In comparison with the striking picture exhibited by the seminal vesicles, the other organs did not show much. There was no spermatogenesis in any of the animals. The ductus deferens did not show a characteristic reaction. The number of mitotic figures in the spermatogenes increased with the increase in size of the seminal vesicles.

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FACTORS WHICH INFLUENCE THE FLOW AND PROTEIN CONTENT OF SUBCUTANEOUS LYMPH IN THE DOG

I. Hemorrhage and Hyperemia*

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Examination under different physiological conditions of the flow and protein content of lymph from the subcutaneous areas of the body is an important method of studying the nature of the tissue fluid and of measuring the interchange of fluid and protein between the capillaries and the lymph (Drinker and Field, 1931).

Although the literature contains a number of observations on the lymph flow from the thoracic duct, comparatively few (table 1) are to be found on lymph formed in the subcutaneous regions where the effects of digestion and respiration are relatively unimportant. While it is impossible to determine the total delivery of lymph in such areas, relative changes in lymph flow may be judged by cannulating a single large lymphatic vessel. Most previous observations of this kind include studies of the effect of active and passive hyperemia on the lymph flow (table 1). Paschutin (1872) and Emminghaus (1873) observed no increase in the lymph flow in the leg of the dog after active hyperemia due to nerve section, although the latter obtained a greater quantity of lymph after venous obstruction. Jankowski (1883) and Rogowicz (1885) later found an increase in the flow of lymph from the leg after cutting the sciatic nerve.

Unpublished observations by Field and Drinker have shown that in the development of a sterile inflammation due to heat the first phase of active hyperemia is accompanied by a slight increase in lymph flow and lymph protein. An extremely large increase in both of these is seen when the temperature of the water in which the foot is immersed reaches 60°C. At this point actual capillary injury apparently begins.

Because of lack of previous data a study has been made of the flow and protein content of lymph after hemorrhage. Observations have also been obtained after active hyperemia under conditions which allowed a definite and measurable increase of the arterial pressure and the blood flow to a part from which lymph was being collected. It was thought possible in this way to learn more about the passage of fluid and protein through the walls of the blood capillaries and lymphatics.

*Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Radcliffe College.

METHOD. Young dogs (15 to 27 kgm.), under Pentobarbital-Sodium, "Nembutal" (sodium-ethyl (1-methyl-butyl) barbiturate), anesthesia were used. The lymphatics accompanying the superficial veins at the ankle were cannulated. At the point chosen for cannulation two lymphatic trunks run beside the veins so that one may be tied and the other cannu-

TABLE 1

Conditions affecting the flow of subcutaneous lymph

ANIMAL	CONDITIONS OF EXPERIMENT	OBSERVER	YEAR	KIND OF LYMPH	CONDITIONS STUDIED	LYMPH FLOW		REMARKS
						Normal	After procedure	
						cc./10 min.	*cc./10 min.*	
Dog	Massage. Opium	Emminghaus	1873	Hind leg	Denervation (tibial and sciatic)	0.39	0.35	
					Venous obstruction by a ligature around the leg	0.39	0.69	
					Venous obstruction directly on the vein	0.39	1.12	
Dog	Morphine. Passive motion and rest	Jankowski	1883	Hind leg	Denervation (sciatic)	3.73*	5.95	
					Turpentine inflammation			
Dog	Morphine. Curare	Rogowicz	1885	Hind leg	Denervation (sciatic)	0.15	0.40	
					Sciatic stimulation	19 mm.	14 mm.	
					Stimulation of vagi	46 mm.	75 mm.	
Dog	Passive motion	Winternitz	1895	Hind leg	Inflammation (turpentine)	0.97	0.96	One experiment only. Study of cell counts
Dog	Curare and opium. Mechanical exercise	Paschutin	1872	Foreleg	Denervation (medulla and cord)	1.08	0.80	
Dog	Nembutal or barbital. Massage	Field and Drinker	1931	Neck	Venous obstruction	0.72		
					Plasmapheresis			
					Sterile inflammation			
Horse	Permanent fistulas	Hamburger	1894	Neck	Pressure on jugular vein	2.88	7.00	
					Pressure on carotid artery	2.88	1.67	
					Feeding	2.88	8.79	
					Work	2.88	8.19	

* Irregular.

lated. In order to obtain a steady flow of lymph, the feet were kept in passive motion by loosely attaching the toes to a wheel which revolved approximately forty times per minute. In some experiments lymph was also obtained by massage from the large lymphatics draining the side of the head and neck. The lymph flowed into calibrated cannulas and was

removed with capillary pipettes at 10-minute intervals. The protein concentration was measured by a Zeiss refractometer, the same observer

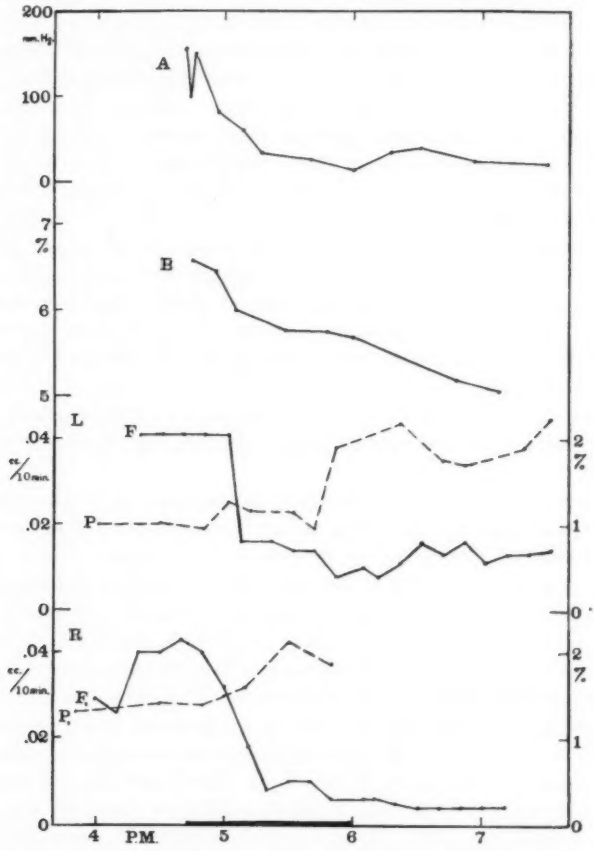

Fig. 1. The effect of hemorrhage on the arterial blood pressure, plasma protein, lymph flow and protein content of leg lymph. *A*, carotid blood pressure in millimeters of mercury; *B*, per cent protein in the blood plasma; *F* and F_1, lymph flow in cubic centimeters per 10 minutes in the left (L) and right (R) forelegs; *P* and P_1, per cent protein in lymph from the left (L) and right (R) forelegs. The solid line on the abscissa represents the time over which 1100 cc. of blood were removed.

making all the readings. In many experiments the blood pressure, protein content of the serum, and skin temperature of the foot were also followed.

Hemorrhage. In the first three experiments of this series, blood amounting to 1.7 to 2.7 per cent (380 to 625 cc.) of the body weight was removed

during a period of 5 to 10 minutes. The arterial pressure fell to about one-third of its normal value and recovered gradually during the next 2 to 3 hours. In the last three experiments, 100 to 200 cc. of blood were removed at intervals of from 10 to 30 minutes until 600 to 1100 cc. had been taken.

Figure 1 displays a typical experiment after severe hemorrhage. From a dog weighing 22 kgm., 1100 cc. of blood were removed in 200 cc. amounts over a period of one and one-quarter hours. Almost immediately after hemorrhage began the plasma protein, blood pressure and lymph flow fell markedly. The protein content of the lymph rose to a peak and then remained above the initial level. Similar changes were observed in all experiments of this series.

Active hyperemia. Since results in the literature on active hyperemia due to nerve section are inconsistent, a small group of experiments was performed in which the flow and protein content of leg lymph were determined before and after tying or cutting the saphenous and sciatic nerves. The skin temperature was measured by a thermocouple attached to the shaved surface of the foot.

Although a rise in skin temperature indicated a slight active hyperemia, no definite change in the flow of lymph nor its protein content was to be observed.

In order to obtain a definitely controlled increase in blood pressure and blood flow through the part from which lymph was collected, a perfusion pump of the type described by Richards and Drinker (1915) was used (fig. 2). The reservoir, *10*, of the pump was filled continuously from the bottom with blood from the carotid artery. Details of the construction of the pump and valves are given in the paper of Richards and Drinker (1915). A membrane manometer was so placed that it would be connected to record either the normal blood pressure of the opposite leg or the pressure delivered by the pump to the perfused leg. Blood was pumped into the artery of the leg either at the ankle or the knee and returned through the animal to the venous reservoir. Aeration of the blood was thus obtained through the normal breathing of the dog. Before perfusion, heparin was injected through a cannula in the jugular vein. Heparin as received from the makers varies in its effects, some specimens lowering blood pressure seriously. The results reported are with non-toxic heparin which was shown during a control period to have no effect on the blood pressure and the flow and composition of lymph. Lymph was collected from the leg: *1*, during a normal period; *2*, after the injection of heparin; *3*, during perfusion at a pressure equal to the normal pressure, and *4*, during perfusion at an increased pressure. In some experiments the sciatic nerve was tied.

Figure 3 shows graphically the results of a typical experiment of this series. The arterial pressure was increased from 110 to 230 mm. of mercury with slight effect on the flow and protein content of the lymph.

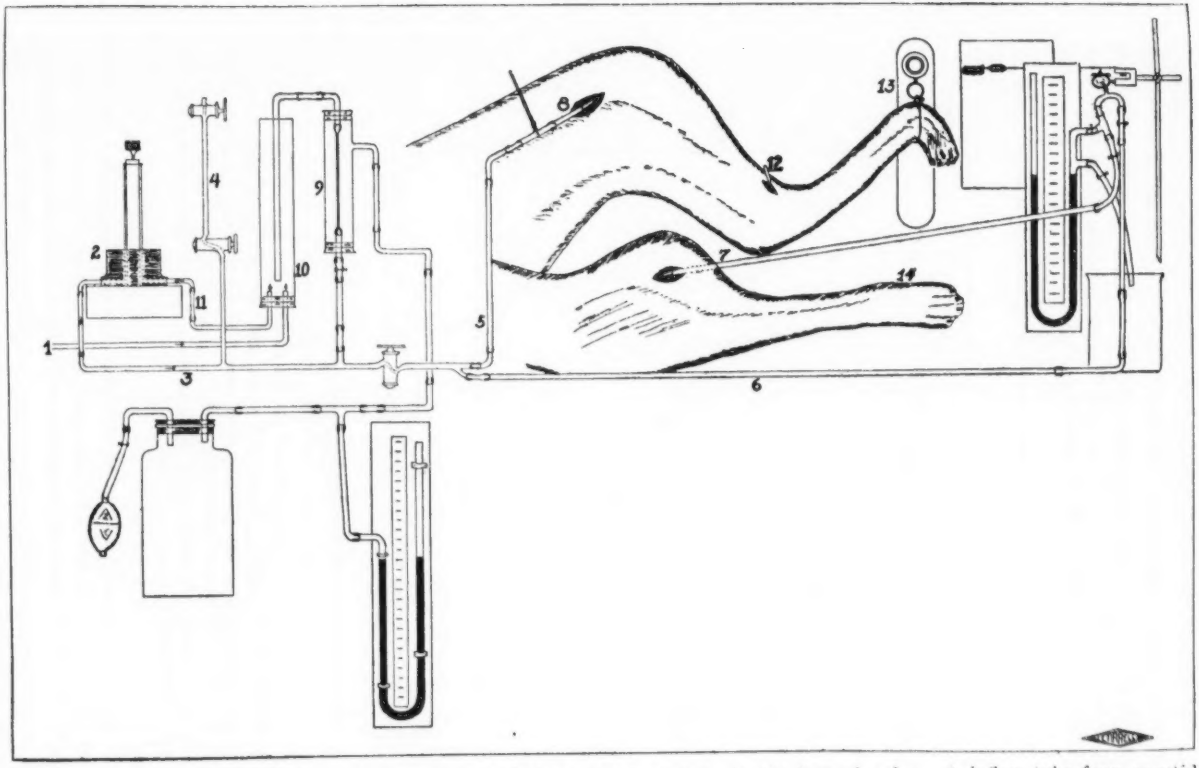

Fig. 2. Apparatus for perfusion and measurement of the arterial pressure in the legs of a dog. *1*, inflow tube from carotid artery; *2*, pump; *3*, outflow from pump; *4*, variable elasticity; *5*, inflow to leg; *6*, pump pressure connection; *7*, arterial blood pressure connection; *8*, arterial cannula; *9*, capillary resistance, adjustment for excess pressure; *10*, venous reservoir; *11*, inflow to pump; *12*, lymph cannula; *13*, foot attached for passive motion; *14*, opposite leg for measurement of normal blood pressure.

Tying the sciatic nerve at this increased pressure produced little change. A further increase of pressure to 290 mm. immediately caused an increased flow of lymph. In another experiment of this group, although the arterial pressure at the knee increased from 130 to over 300 mm. of mercury and the blood flow from 100 to 250 or 300 cc. per minute, the flow of lymph de-

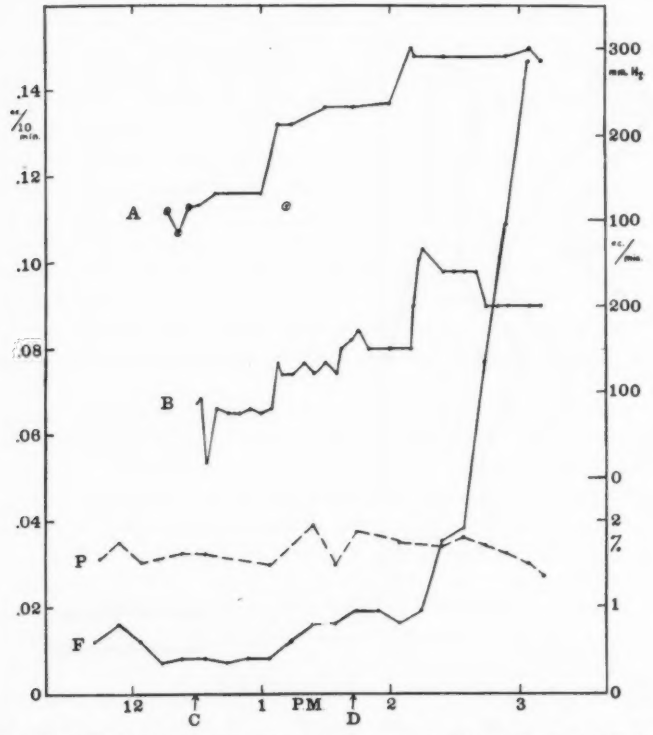

Fig. 3. The effect of increased arterial pressure and blood flow on the flow and protein content of leg lymph. *A*, arterial blood pressure of leg in millimeters of mercury (circles represent the arterial pressure without perfusion); *B*, blood flow through the leg in cubic centimeters per minute; *P*, per cent protein in lymph; *F*, flow of lymph in cubic centimeters per 10 minutes. At *C* perfusion of the leg was begun and at *D* the sciatic nerve was tied.

creased slightly and remained at a low level for over an hour. At this time partial venous obstruction was produced by tying a piece of rubber around the leg above the lymph cannula. Within ten minutes the flow had begun to increase and within a short time the protein content of the lymph decreased. This indicates, as have other experiments, that venous ob-

struction is more effective in causing an outpouring of fluid than increasing arterial pressure. In no case, whether the lymph flow increased or not,

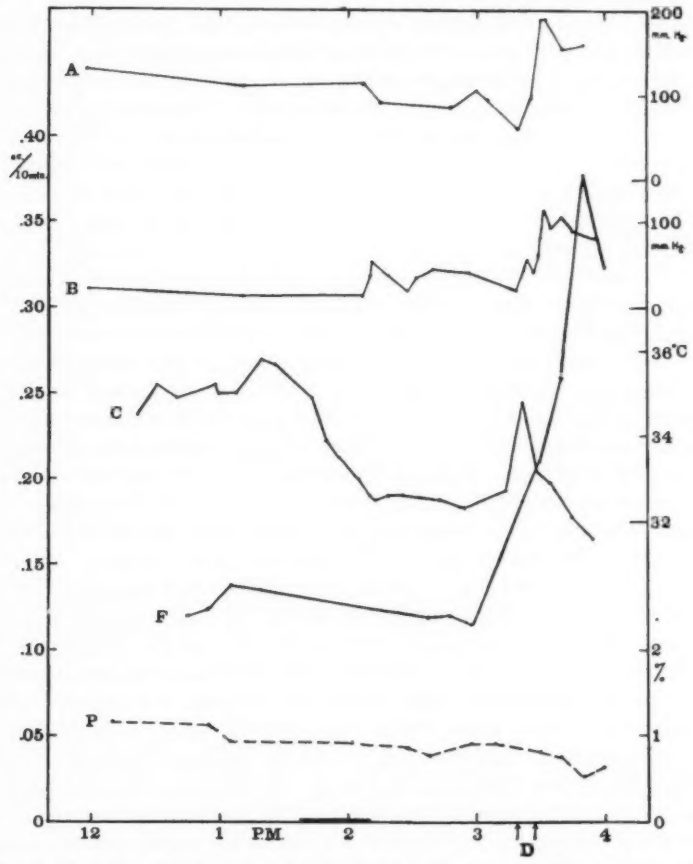

Fig. 4. The effect of arterio-venous anastomosis on the arterial and venous blood pressures, skin temperature and the flow and protein content of leg lymph. *A*, carotid blood pressure in millimeters of mercury; *B*, venous pressure (in millimeters of mercury) just above the lymph cannula; *C*, temperature of the skin of the foot; *F*, lymph flow in cubic centimeters per 10 minutes; *P*, per cent protein in lymph. The solid line on the abscissa represents the time taken to complete the anastomosis. At *D*, 90 mgm. of ephedrine were injected intravenously.

did the protein content of the lymph change significantly with an increase in arterial pressure.

In a later experiment (fig. 4), the venous pressure of the leg was increased

by an anastomosis between the femoral artery and vein. The venous pressure was measured at the ankle by a venous cannula which lead to a mercury manometer. The skin temperature was used as a criterion of the amount of blood going through the capillaries of the foot. After arteriovenous anastomosis the lymph flow and protein content were unchanged, but the arterial blood pressure and the skin temperature were low and the blood flow through the foot was probably slight. Pulsations could be felt in the part of the vein proximal to the anastomosis and a definite bruit could be heard over the veins at some distance below the union. When the arterial pressure was then increased by ephedrine the lymph poured out. At this time the protein fell slightly, showing that water was forced out faster than protein.

Discussion. After hemorrhage the fluid and protein of the blood are quickly replaced. Hirota (1928) showed that if the hemorrhage is not too severe, more than 50 per cent of the amount of fluid lost is restored from the tissue fluid, usually in about half an hour. The protein of the blood has been shown by Morawitz (1906) to be made up to about half its normal value in the first three hours. As would be expected from such an inflow of fluid into the blood capillaries, the flow of lymph is decreased and the protein content increased. Thus, in hemorrhage as in venous obstruction and plasmapheresis, the lymph rapidly reflects changes in the tissue fluid. The amount of protein in the lymph does not appear large enough for any significant return of protein to the blood via the subcutaneous lymph. Calculations based on figure 1 show that the amount of protein coming out in the lymph per unit of time is about half its normal value after hemorrhage.

From a series of experiments on thoracic duct lymph, Starling (1894) concluded that intracapillary pressure is the chief factor in lymph production. In our experiments when the arterial pressure was altered through an isolated part from which lymph was collected, results indicate that arterial pressure and blood flow may be greatly increased without changing the flow and protein content of the lymph. Denervation has little effect on these results. This must be explained by the assumption that the resistance of the arterioles is sufficient to prevent undue augmentation of capillary pressure. It is only when the arteriolar resistance gives way under excessive pressure that the lymph flow suddenly increases. It may be assumed that venous obstruction was more effective in increasing capillary pressure and therefore lymph flow than arterial pressure. This observation agrees with Rous' idea of a gradient of permeability toward the venous end of the capillary. McMaster and Hudack (1932) have recently shown that this gradient is accentuated and broadened in scope by moderately increasing the venous pressure, so that transudation takes place abundantly from the venules. The permeability of the portion of the capil-

lary web near the arterioles is increased only when venous pressure approximates that in the arteries.

The fact that arterio-venous anastomosis did not increase the lymph flow may probably be accounted for by the fact that blood was shunted back by the vein and the foot was poorly supplied with blood. This is indicated by the low level of the skin temperature. When the arterial pressure was sufficiently increased by ephedrine so that the blood flow was resumed and the effect of increased venous pressure alone was obtained, the lymph flow increased.

SUMMARY

1. Hemorrhage decreases the flow of subcutaneous lymph and increases its protein content.

2. Active hyperemia due to arterial perfusion of the dog's leg under increased pressure has little effect on the lymph from the part until the arterial pressure has become nearly three times its normal value.

The writer wishes to express her gratitude to Dr. Cecil K. Drinker, who suggested the problem on which this work is based, for advice and help, as well as to Dr. Madeleine E. Field for assistance in performing the experiments.

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FURTHER OBSERVATIONS ON THE RAPIDITY OF PASSAGE OF SUBSTANCES FROM BLOOD TO LYMPH IN THE DOG*

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Observations on subcutaneous as well as thoracic duct lymph show that many substances pass rapidly from the blood to the lymph. Rous and Gilding (1929) injected a very diffusible dye, brom phenol blue, intravenously in rabbits and cats over a period of one minute and found the mesenteric lymphatics distended with blue fluid within 15 seconds after injection, a vivid expression of the great permeability of the capillaries in this region and of the ease with which material in the tissue spaces reaches lymphatics. Less diffusible dyes, as trypan blue and vital red, injected intravenously in dogs, have been observed in the thoracic duct lymph after 8 to 12 minutes (Meyer-Bisch and Lampe, 1924; Keith, Rowntree and Geraghty, 1915; and Harris, 1920). Smith (1925) was able to detect vital red in the thoracic duct lymph within 2 to 3 minutes after intravenous injection.

Substances of larger molecular weight have also been found to pass rapidly from the blood into this lymph. Petersen, Levinson and Hughes (1923) observed that injected hemoglobin went from the blood to the thoracic duct lymph in 12 to 15 minutes. Osato (1921) states that true solutions as well as colloids (indigo carmine, phenolsulphonephthalein, foreign protein and antibodies) introduced into the blood regularly appear in the thoracic duct lymph in 4 to 5 minutes after injection. Field and Drinker (1931a, b) working on lymph from subcutaneous lymphatics of dogs showed by intravenous protein injections as well as by experiments on sterile inflammation, venous obstruction and plasmapheresis, that subcutaneous lymph promptly reflects alterations in the constituents of the serum. In none of these experiments, however, have accurate measurements been made of the time taken for substances to pass from the blood stream into the subcutaneous lymph. Such measurements are significant in comparison with determinations on the thoracic duct lymph since the latter comes largely from the liver and intestines, the capillaries of which are known to be exceptionally permeable to protein.

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Many of the experiments on lymph formation now in progress in this laboratory have shown a very rapid equalization of concentration of substances between blood and lymph. The work of Rous and his associates has provided data on the rate at which dyes of different degrees of diffusibility leave the capillaries. The time elapsing between their appearance in the capillaries and in the subcutaneous lymph has been considered of importance in indicating the degree of extravascular circulation which is constantly in progress. In order to measure this time a very diffusible dye, brom phenol blue, has been employed, and the interval between intravenous injection, the first coloration of the skin indicating capillary

TABLE 1

Passage of substances from blood to subcutaneous lymph

SUBSTANCE	DOSE	MOLECULAR WEIGHT	KIND OF LYMPH	NUMBER OF DETERMINATIONS	TIME OF APPEARANCE IN LYMPH	AVERAGE TIME	REMARKS
					minutes	*minutes*	
Brom phenol blue	45 to 55 cc. of 3 to 4 per cent solution	670	Cervical	3	2.1 to 3.0	2.7	Skin and tissue appeared blue in one-half minute
			Foreleg	3	3.5 to 9.0	6.3	
			Hind leg	3	4.0 to 11.0	6.5	
			Mesenteric	1		2.5	
Vital red	50 cc. of 2 per cent solution	1,096	Cervical	3	1.5 to 3	2.2	One experiment after pitressin and one after ephedrine
			Hind leg	2	4.5 to 11	7.8	
Egg albumen	100 cc. of 5 ± per cent solution	34,500 (Svedberg and Nichols, 1926)	Cervical	4	9 to 14	12	
			Hind leg	3	11 to 13	13	
Hemoglobin (dog)	100 cc. of 6.5 ± per cent solution	66,800 (Svedberg and Fåhreus, 1926, horse Hb)	Cervical	4	37 to 48	44	
			Foreleg	1	32	32	

escape, and the appearance in cannulated lymphatic trunks has been recorded. To obtain an idea of the speed with which substances of different molecular weights pass into the lymph, similar experiments have been performed with a slowly diffusible dye and with two proteins of widely different molecular size.

METHODS AND RESULTS. The dogs used were anesthetized with Pentobarbital-Sodium, "Nembutal" (sodium-ethyl (1-methyl-butyl) barbituate), intraperitoneally. The lymphatics of the lower leg and of the cervical region were cannulated as previously described (Haynes, 1932). Substances such as brom phenol blue,[1] vital red and hemoglobin were injected

[1] A specially purified product obtained from Hynson, Westcott and Dunning.

into the jugular or femoral vein, and the lymph collected from the cannulas in capillary tubes every one-half or one minute thereafter. By comparing such tubes with tubes of normal lymph the first trace of color could be observed. Egg albumen could be accurately detected in lymph by a micro precipitation test.[2] Usually slight pressure below the tip of the cannula was necessary to force lymph in the lymph vessel into the cannula. The arterial blood pressure and the protein content of the lymph were measured before and after injection.

Brom phenol blue was found to be non-toxic when the hydrogen ion concentration was adjusted to that of the blood. Vital red, hemoglobin and egg albumen injections were also innocuous as judged by the constancy of the arterial blood pressure and of the protein content of the lymph. Lymph flow did not change significantly.

The results, summarized in table 1, show that dyes such as brom phenol blue and vital red appeared in lymph from the neck in approximately 2½ minutes and in the lymph from the legs in 6 to 8 minutes. The skin and mucous membranes appeared blue in less than 30 seconds.

Egg albumen could be detected in the cervical lymph in 9 to 14 minutes after the beginning of injection and in the lymph of the hind leg in 13 minutes. Hemoglobin, although much more difficult of detection, appeared in the subcutaneous lymph from 30 to 45 minutes after injection.

Discussion. The data in table 1 indicate that the time required for the passage of substances into the lymph is increased with an increase in the molecular weight of the substance. The great difficulty of detecting the color of hemoglobin in the lymph probably accounts in part for the relatively late appearance of this material.

The use of vital red is significant since Harrop and Waterfield (1930) have claimed that in the dye method of measuring the blood volume part of the vital red diffuses into the lymph spaces, thus making the method less accurate than the other methods. The present experiments confirm the observations of Smith (1925) on thoracic duct lymph and make it obvious that the dye must begin to pass out of the blood capillaries almost immediately.

Of the total times determined in these experiments, the time for the injected substance to be carried by the blood to different parts of the body may be considered roughly as of the order of 10 to 20 seconds. We observed that the skin and mucous membranes were diffusely stained 30 seconds after the beginning of an injection of brom phenol blue. It may thus be considered that dissolved substances in the tissue spaces may get into small lymphatics and be carried to large collecting trunks in the

[2] Anti-egg white rabbit serum of a titre of 1–100,000 was supplied by Dr. Walter Bauer.

legs in less than 6 minutes. In the neck region the time is only about 2 minutes.

SUMMARY

NON-TOXIC SUBSTANCE INTRAVENOUSLY INJECTED	NATURE OF SUBSTANCE	AVERAGE TIME OF APPEARANCE IN	
		Cervical lymph	Leg lymph
		minutes	*minutes*
Brom phenol blue	Very diffusible dye	2.7	6.4
Vital red	Slowly diffusible dye	2.2	7.8
Egg albumen	Protein of relatively small molecular weight	12	13
Hemoglobin	Substance of large molecular weight	44	32

These experiments as well as experiments on substances such as histamine, intravenously injected, also suggest a rapid interchange of material between blood and subcutaneous lymph.

The writer wishes to express her gratitude to Dr. Cecil K. Drinker and to Dr. Madeleine E. Field whose advice and assistance were invaluable during the course of these experiments.

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THE ACCUMULATION OF LACTIC ACID IN EXCISED LIVER TISSUE

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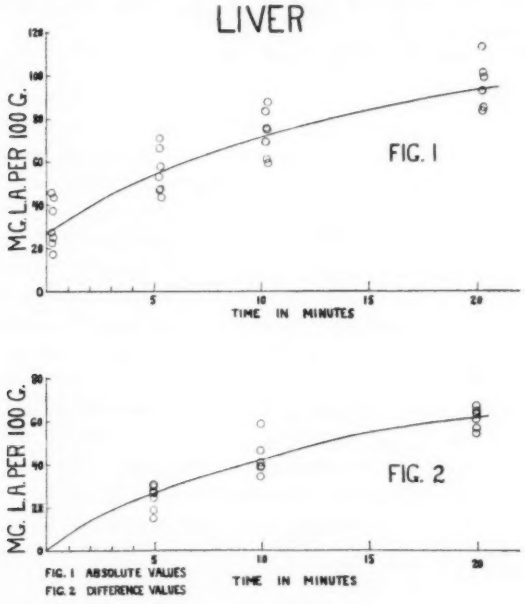
The initial lactic acid content and the rate of lactic acid accumulation in excised dogs' liver during a twenty minute period have been determined as supplementary data to a previous report (Haldi, 1932) on the accumulation of lactic acid in excised brain, kidney, muscle and testicle. The procedure was essentially the same as in the former experiments. Immediately after decapitation of the animal one lobe of the liver was quickly removed and a portion of the tissue, approximately 3 to 6 grams, sliced off and plunged into liquid air. Fifteen to eighteen seconds elapsed from the moment the blade of the guillotine struck the animal's head until the tissue was immersed in liquid air. Three other pieces of tissue of approximately equal weight were taken from the same lobe, incubated 5, 10 and 20 minutes respectively at 38°C. and then frozen in liquid air.

The results of the experiments are represented in figures 1 and 2. The initial lactic acid content in 7 experiments varied between 17.1 and 45.7 mgm. per cent with an average of 31.3 mgm. per cent. After 5, 10 and 20 minutes' incubation the liver contained an average of 54.7, 73.0 and 95.6 mgm. per cent respectively.

The increase in lactic acid was determined by subtracting the initial content in milligrams per cent from the amount present after incubation. The average increase in lactic acid content after 5, 10 and 20 minutes' incubation of the tissue was 23.4, 41.7 and 64.3 mgm. per cent.

In one experiment unusually high values were obtained which are not represented in the figures. The initial lactic acid content was 63.2 mgm. per cent and at the end of 5, 10 and 20 minutes' incubation the tissue contained 96.9, 129.6 and 154.0 mgm. per cent or an increase of 33.7, 66.4, and 90.8 mgm. per cent respectively. These values were omitted in constructing the curves because of the conditions of the experiment. The animal was extremely vivacious and when brought to the laboratory continuously ran around the room in a lively manner for several minutes before it was decapitated. When brought up to the guillotine it had to be forcefully restrained because of its efforts to break loose from the attendant. It was therefore thought that the high lactic acid values might not represent normal variations but might have been due to the activity of the animal.

Comparison of the curves in figures 1 and 2 reveals a wider variation in the absolute values at various intervals than in the difference values. This might possibly be explained on the basis of the observations by Himwich, Koskoff and Nahum (1928, 1929) that the liver removes lactic acid from the blood. The lactic acid concentration of the blood, and, in all probability, the functional activity of the liver varies with different animals. On the plausible assumption that various livers contain a different amount of lactic acid absorbed from the blood, we might expect to find wide variations in the initial lactic acid content of the liver which would be maintained



in the absolute values throughout the experiment. These variations however would not show up in the curve of difference values which represents only the rate of lactic acid formation in liver tissue.

SUMMARY

The initial lactic acid content and the rate of lactic acid accumulation in excised dog's liver has been determined.

The initial content (15 to 18 seconds after decapitation) varied between 17.1 and 45.7 mgm. per cent with an average of 31.3 mgm. per cent.

The average increase was 23.4, 41.7 and 61.3 mgm. per cent after 5, 10 and 20 minutes' incubation.

Unusually high values were obtained in one experiment. Owing to the conditions of the experiment it is impossible to decide whether these values represented a normal variation or should be attributed to the activity of the animal previous to decapitation.

The absolute values showed wider variations than difference values. An explanation is suggested on the basis of the observations that liver tissue absorbs lactic acid from the blood.

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PREVENTION OF "CASTRATION CELLS" IN THE ANTERIOR PITUITARY OF THE MALE RAT BY ADMINISTRATION OF THE MALE SEX HORMONE

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Since Biedl (1912) reported the discovery by Zacherl of the castration cell in the anterior hypophysis of the rat, numerous articles of a corroboratory nature have appeared until now the phenomenon is a matter of common knowledge. That the "castration cell" was not an entirely new cell type, however, was later established by Addison (1917) who showed that it is really the basophile normal to the anterior lobe which has undergone a characteristic modification after gonadectomy. He states that the castration reaction is discernible at the end of the first week.

That castration changes in the anterior hypophysis might be inhibited by substitution of the endocrine principles from the gonads has occurred to several workers. Fichera (1905) thought that the hypophyses of three capons that he had treated with saline extracts of cock testes approached the normal. Schleidt (1914), upon investigation of the hypophyses of Steinach's feminized male and masculinized female rats, reported that heterologous implants prevented castration changes in that form. Nukariya (1926) stated that in castrated rats treated with saline extracts of the rat epididymis and testis the basophiles typical of castration were somewhat less numerous than in the untreated animals castrated for a like period. Lehmann (1927) describes inhibition of the castration picture in castrated rats implanted with rat and human gonads and also those treated with extracts of human gonads. Fluhmann and Kulchar (1931) were unable to prevent the development of castration cells in the female rat by the use of "amniotin (Squibb)."

MATERIAL AND METHOD. The work of Moore, Gallagher, Koch, and others has yielded the male sex hormone in a rather highly purified state. The fact that the preparations of these workers have undergone far more than a cursory study and that they may be standardized by four methods (Moore, Hughes and Gallagher, 1930) suggested to us that a study of the effect of these extracts on the rat pituitary in castration might prove of much interest. Through the courtesy of Doctor Gallagher a small amount of extract derived from male urine (method as yet unpublished) and of

proven potency, was made available for this study. This material containing 17½ bird units per cubic centimeter was injected subcutaneously in doses of 0.25 cc. twice daily into two adult castrated male rats. Injection started immediately after castration and was continued for twenty days.

Following essentially the method of Gallagher and Koch (1929), we undertook to prepare an extract of bull testes. The method used may be briefly outlined as follows: The ground testicular tissue[1] was extracted with four volumes of 95 per cent ethyl alcohol, the ground tissue filtered off, and the filtrate evaporated in vacuo. The residue was extracted with benzene, the precipitate discarded, and the benzene evaporated in vacuo. The residue from the benzene was extracted with acetone, the acetone evaporated, and the acetone-soluble fraction dissolved in petroleum ether. The petroleum ether preparation was then shaken in a separatory funnel with a 70 per cent alcohol solution. The 70 per cent alcohol fraction was concentrated and taken up in ether; the ether was then evaporated and the ether-soluble material taken up in olive oil. Injections of this preparation in 0.25 cc. doses twice daily for twenty days were given to five adult castrated male rats. Six other castrated male animals of the same age were used as controls. Two of these were injected with corresponding doses of the aqueous fraction which had been separated from the ether-soluble material in the last step. Another rat was injected with equal doses of the same olive oil as had been used for dissolving the ether-soluble material. Four rats of the same age were kept as normal controls.

In the series of rats given bull testes extract, treated castrates, castrate controls, and normals were fixed in the same container, carried through all procedures together, and finally mounted on the same slides. The histological methods employed for all hypophyses may be described briefly: After fixation in Helly's fluid for one hour at a temperature of 35° to 37°C., the gland is washed in distilled water, dehydrated as far as 70 per cent alcohol and left overnight. Dehydration must then be completed, after which the tissue is cleared in oil of bergamot and imbedded in paraffin.

Plate I. Explanation of figures. Photomicrographs of anterior hypophyses of adult male rats. Preparations were fixed in Helly's fluid and stained with orange G and aniline blue (× 600).

1. Anterior hypophysis of rat castrated for twenty days.
2. Anterior hypophysis of normal untreated rat. Ink outlines denote cells shown in figures 5 and 9.
3. Anterior hypophysis of twenty-day castrated rat receiving injections of human male urine extract during the twenty days after castration.
4. Anterior hypophysis of twenty-day castrated rat receiving injections of bull testes extract during the twenty days after castration. Ink outlines denote cells shown in figure 6.

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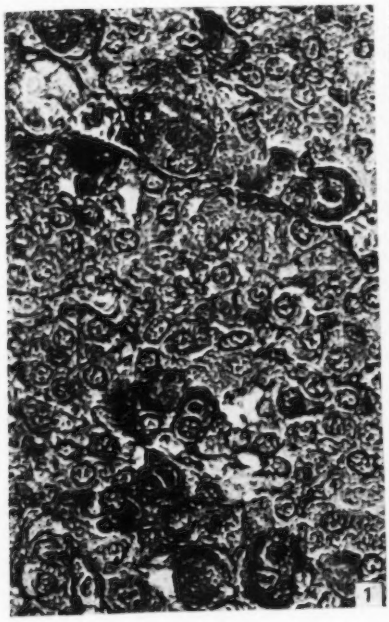
[1] Approximately 15 kilograms of fresh bull testes were used.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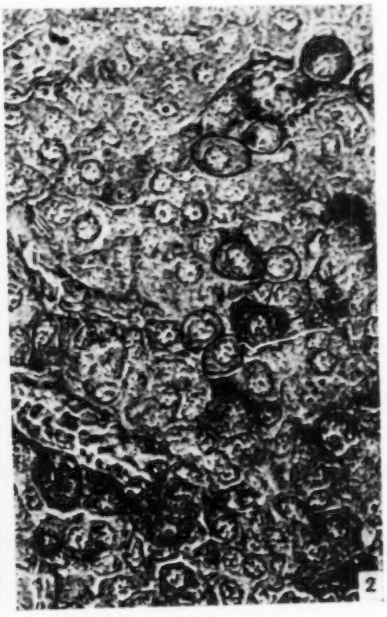
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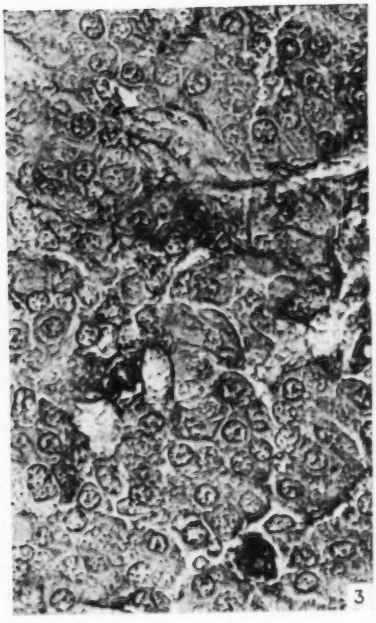
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Serial sections are cut at 7.5 micra. As it has been the experience in this laboratory that hematoxylin-eosin preparations are entirely unsatisfactory from the standpoint of cell differentiation in the rat hypophysis, we have resorted to a modification of the Mallory orange G-aniline blue technique which has proved satisfactory. Details of the technique are as follows: After going through xylol and the higher concentrations of alcohol, the slide is left 90 minutes in iodized 70 per cent alcohol to remove mercuric chloride crystals. It is then passed through alcohols of decreasing concentration to water and thence to a slightly acidified solution of two per cent aqueous orange G where it remains for 20 minutes. The slide is drained of excess dye and placed in one per cent phosphomolybdic acid for four minutes when it is again drained. Then for a period of 12 seconds the slide is moved back and forth in a five-tenths per cent aqueous solution of aniline blue. Next it is rinsed in 95 per cent alcohol, dehydration completed in 100 per cent alcohol and after xylol clearing, the tissue is mounted in xylol balsam.

RESULTS. In order that the following experimental results may be enentirely comprehensible we may here briefly summarize the changes in the rat hypophysis during the first three weeks after castration. At the end of the stated period one finds in the castrate pituitary a marked increase in the number of basophiles. In addition to basophilic cells not exceeding the normal in size and depth of staining, one sees cells which exceed normal diameters. In general, these large cells fall into two classes. The very largest cells stain lightly with aniline blue. The smaller ones take a deeper stain and possess an exceedingly prominent macula which is usually central (figs. 1 and 8). The prominence of the macula, *m*, is characteristic of the basophile after castration. In the hypophysis of the normal rat the macula is not as often seen in the basophile and its prominence in the cytoplasm is by no means as well marked as after gonadectomy.

Previous to this experiment Reese studied the cell types in the anterior

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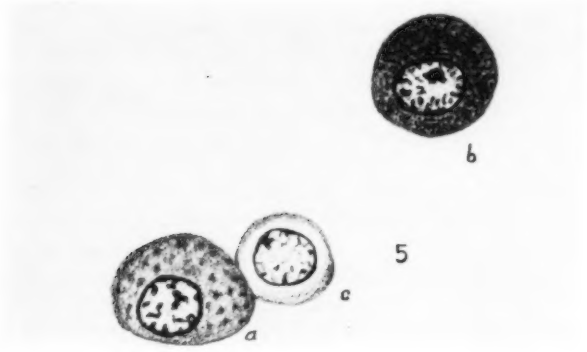
Plate II. Explanation of figures. Camera-lucida drawings of cells of anterior hypophyses of adult male rats. Preparations were fixed in Helly's fluid and stained with orange G and aniline blue. Abbreviations: *a*, acidophile; *b*, basophile; *b'*, large deeply staining basophile with prominent macula, *m*, typical of twenty-day castrated rats; *b''*, very large lightly staining basophile typical of twenty-day castrasted rats; *m*, macula; *c*, chromophobe. (× 1600)

5. Anterior hypophysis of normal untreated rat. Group of cells marked on right upper corner of figure 2, plate I.

6, 7. Anterior hypophyses of two twenty-day castrated rats receiving injections of bull testes extract during the twenty days after castration. Figure 6 shows the group of cells marked on figure 4, plate I.

8. Anterior hypophysis of rat castrated for twenty days.

9. Anterior hypophysis of normal untreated rat. Group of cells outlined in center of figure 2, plate I.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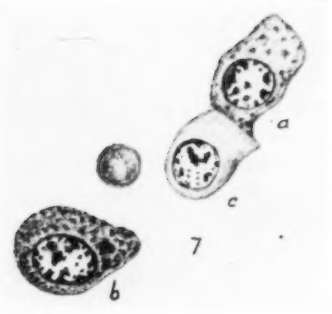
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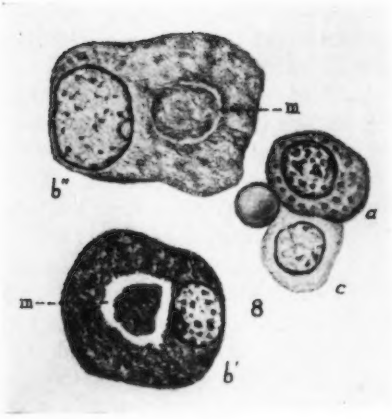
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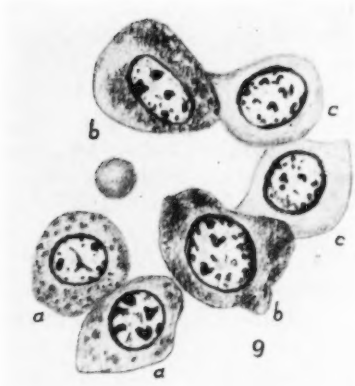
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pituitary of the female rat after castration, and reports having found that in the female there is approximately a threefold increase in the number of basophiles twenty days after castration. His work was based on differential counts of more than 4000 cells made on representative fields in the pituitaries of both normal and castrate types.

In the present experiment where only male rats were under consideration, cell counting was undertaken by McQueen-Williams for the purpose of determining the relative number of the three cell types prevailing in typical fields in the hypophyses of normal and castrate animals. These fields were not picked at random but were those that seemed to express the predominant characteristic of the sections studied. The total number of cells counted for each group of animals amounted to over 10,000. The results would seem to indicate that in the case of the adult male rat there is almost a twofold increase in the number of basophiles in the hypophysis twenty days after castration.

The two animals which were injected with the urine material from Doctor Gallagher's laboratory showed in the seminal vesicles the picture given by Moore, Hughes and Gallagher (1930). In these treated castrates the involution of the secretory epithelium of the seminal vesicle and anterior lobe of the prostate incident to castration was minimal and this epithelium was in no way comparable to that of the twenty day castrate. Similar results were obtained in the case of the rats treated with bull testes extract. An interesting fact pertains to the relative weights in this last series: The average weight of the seminal vesicle in the castrates was 140 mgm. as compared with 636 mgm. for the treated castrates and 710 mgm. for the normal rats.

A study of the anterior lobe of the pituitary in the two rats treated with the urine derivative showed in this gland a marked absence of the basophile so typical of the castrate animal. For comparison of the two conditions see figures 1 and 3. The basophiles are smaller and far less numerous in the injected animals than in the controls castrated for the same period, and the prominence of the macula and its position in the central portion of the cytoplasm which is so characteristic of the castrate is seldom observed in the treated castrate and is likewise rare in the normal.

The five castrates treated with the ether-soluble fraction from the bull testes exhibited a picture in the hypophysis the principal aspects of which were identical with those treated with the urine material. Here again the basophile changes typical of castration were not to be found (figs. 4, 6 and 7). On the other hand, controls injected with the aqueous residue after ether extraction of the alcohol-soluble fraction in amounts volumetrically equivalent to the lipoid-soluble bull testes extract showed marked castration changes. This was also true of the animal injected with olive oil alone.

CONCLUSION

The morphological changes in the anterior lobe of the male rat hypophysis incident to castration may be prevented by the administration of extracts containing the active principle of the male gonads. The pituitary gland of the castrate male rat may be similarly affected by treatment with a derivative of human male urine.

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THE ANTERIOR PITUITARY SEX HORMONE IN THE BLOOD AND URINE OF RATS

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It is now well established in a few species of animals that during pregnancy the anterior pituitary sex hormones, or hormones with similar action, are secreted in much larger amounts than during the non-pregnant state. This increase in the amount of the hormones in the blood and urine of pregnant animals has been studied especially in women and mares and to some extent in other mammals. It seems that in rats the test is negative during pregnancy (Snyder and Wislocki, 1931). However, in castrated rats the results may be positive according to Zondek (1930) who, in a footnote, calls attention to a positive test he obtained by injecting urine. The present article deals with the anterior pituitary sex hormones in the blood and urine of albino rats in respect to sex and castration.

The test used was similar to those in general use and depends upon an increase in weight of the ovaries and uterus and the appearance of cornified cells in the vagina of immature rats. Young females about 25 days old and weighing 36 to 40 grams were taken from the mother, marked and started on the test. Subcutaneous injections were made twice daily when urine was used, while with blood the morning injection was subcutaneous and the evening injection intraperitoneal. The injections continued for a period of 4 to 15 days. Three or four days after the last injection the animals were killed, the ovaries and uterus, cut from the vagina just anterior to the cervix and with the Fallopian tubes intact, were dissected free from connective tissue and weighed in a closed container. This method of weighing has been used in the laboratory for some time (Emery, Bash and Lewis, 1931) and, therefore, the error in weight due to dissecting out these organs was small.

Blood. During the past two years a large number of rats have been killed for various purposes. These animals with a known history have afforded an excellent opportunity to obtain blood in sufficient amounts to carry out the tests here described. Table 1 shows the effects of serum from three types of donors on the weights of the ovaries and uteri and changes in the vagina of the recipients. It is interesting that the anterior pituitary sex hormones were not found in pregnant rat serum even though as much as

25 cc. was given to an immature rat whereas 14 to 16 cc. of castrated male serum caused the ovaries and uterus to enlarge and the vagina to open in full oestrus. In this respect pregnant rat serum differs from that of pregnant women and mares. The negative results from serum of normal male and female rats previously reported (Emery, Bash and Lewis, 1931) were again confirmed.

Although not shown in table 1, the blood serum of castrated multiparous female rats has been found to be potent. The amount of blood available in this group has been small and, therefore, only a few tests have been made. These tests show that the potency of castrated female serum is similar to that of castrated male serum as shown in table 1. These data would lend support to the view that castration in rats results in a hypersecretion of the anterior pituitary sex hormones and not simply a storage of the hormones in the gland as Evans and Simpson (1929) described. In humans where the ovaries were removed or in certain cases with various menstrual

TABLE 1

Rat serum from three kinds of donors was injected into immature female rats (recipients). Body weight in grams, ovaries and uteri in milligrams.

RECIPIENTS						DONORS
Number of rats	Body weight	Ovaries weight	Uteri weight	Number of vaginas open	Number in oestrus	Average amount and kind of serum injected
20	45	20	60	18	15	19 cc. castrated male
9	50	15	40	None	None	26 cc. normal male
5	48	12	34	None	None	17 cc. pregnant
15	57	15	40	None	None	None (controls)

disturbances, positive results, similar to those here described for rats, have been reported by Fluhmann (1929) and Mazer and Hoffman (1931). In fact in cases of hydatidiform mole and malignant chorionepithelioma the amount of the hormones excreted is greater than that excreted during normal pregnancy (Mack and Catherwood, 1930).

Urine. The rats were placed in a wire cage with a large glass funnel beneath, which drained the urine into a test-tube containing ether. Screen wire in the funnel kept the feces and hair from falling into the test-tube. Toxic substances were further reduced by shaking with ether as described by Böhne (1931). In these experiments one volume of urine was shaken with two volumes of ether, then filtered. The ether in some cases was removed by bubbling air through it at room temperature; in other cases heating in a water bath at 70°C. removed all traces of the ether. This simple method although almost entirely preventing fecal contamination did not remove all the toxic substances from the urine. It was, therefore, necessary to limit the daily dose to 2 or 3 cc. and continue the injections over a

10 to 15 day-period in order to give a total dose of 30 to 40 cc. of urine to each immature rat. Even these large doses gave negative results. Since this is about 10 times the threshold dose for pregnant human urine, it would seem unlikely that larger doses of rat urine given in this way would show positive results. An attempt was, therefore, made to make an extract of the rat urine using the alcohol precipitation method of Frank (1931). The results so far have all been negative even when the extract from 100 cc. or more of urine was given to each immature rat. Extracts made from the urine of pregnant, castrated female and castrated male rats were tried but no evidence of the hormones was found. These urine extracts are often toxic as others also have reported (Hill and Parkes, 1930). These toxic substances and the small amount of urine available are factors which hinder the preparation of the hormone in concentrated form from rat urine.

TABLE 2

Urine from three types of rats (donors) was injected into immature female rats (recipients). Body weight in grams, ovaries and uteri in milligrams. Compare these data to controls, table 1.

RECIPIENTS						DONORS
Number of rats	Body weight	Ovaries weight	Uteri weight	Number of vaginas open	Number in oestrus	Average amount and kind of urine injected
10	48	10	27	None	None	24 cc. castrated male
10	64	14	41	None	None	27 cc. castrated female
8	64	12	44	None	None	28 cc. pregnant

The abundance of the pituitary sex hormones in pregnant human urine (using this rat test, we have obtained positive results with less than 5 cc. of pregnant human urine) is quite in contrast to the findings with rat urine. In table 2 tests from urine of pregnant, castrated female and castrated male rats are given. It is clearly seen that the ovaries and uterus were not enlarged; in fact the weights are slightly below the normals shown in table 1. It was thought probable that toxic substances in the urine may have retarded the growth of the ovaries and uterus as well as body weight. On the other hand, rats while being injected daily with urine readily respond to pituitary grafts, thus showing the urine injections did not retard or noticeably inhibit the action of the anterior pituitary sex hormones when present in amounts large enough to stimulate growth and luteinization of the follicles.

Discussion. The amount of anterior pituitary sex hormones found in the blood serum of castrated male and castrated female rats is considerably less than the amount present in pregnant human serum. Trivino (1926), Siddall (1928), and Fluhmann (1929 and 1930) have reported

positive results in immature and adult mice with 3–5 cc. of pregnant human serum. This compares with 14 cc. of castrated rat serum which is the minimal amount that will produce enlargement of the uterus and the appearance of cornified cells in the vagina of immature rats (table 1). It is interesting that with threshold doses of this hormone, either given as pituitary grafts (Emery, Bash and Lewis, 1931) or injected as blood serum (table 1), the ovaries may increase only a few milligrams in weight whereas the percentage increases in weight of the uterus is considerably more. This seems to show that a small enlargment of the follicles as represented by the increased weight of the ovaries can stimulate a large growth of the uterus. Although the ovaries may be decreased in size accompanied by a positive vaginal reaction (Cole and Hart, 1930), there is no definite proof that the anterior pituitary sex hormones can produce growth of the rat's uterus independent of the ovaries.

Larger doses, 25 to 30 cc., of castrated male serum caused the ovaries of immature rats to enlarge to 100 milligrams or more. These ovaries were full of large corpora lutea and resembled those produced by pituitary grafts. This is further evidence that the ovarian stimulating hormones in the blood of castrated rats are similar if not identical to those of the pituitary gland.

It was disappointing to find that the pregnant serum of rats did not contain the pituitary sex hormones characteristic of some of the other mammals. In addition to the positive results with pregnant human serum cited above Cole and Hart (1930) have discovered in the serum of pregnant mares a most remarkably high concentration of this hormone. They have obtained an increase in the weight of rat's ovaries with as little as $\frac{1}{20}$ cc. of serum; this is about three hundred times the concentration found in castrated rat serum.

A fair amount of the hormone in the blood and lack of it in the urine of castrated rats is interesting because pregnant human urine is loaded with the hormone while pregnant human serum does not seem to be so potent. It was expected that the urine of rats would be found positive for the pituitary sex hormone and that a comparison could be made with the amount in the serum. Such a study would throw light on the threshold of the kidney for this hormone in the several types of rats studied. In some animals the kidney threshold probably does determine to some extent the output of the hormone in the urine (Frank, 1931). Aside from pregnant human urine positive results have been reported from the urine of monkeys by Aschheim (1930). In contrast to this, negative results have been reported in several animals, as cat, dog, rabbit, rat, sow, mouse, cow and elephant (Aschheim, 1930; Leonard, 1931; Snyder and Wislocki, 1931). These variations of the hormone occur not only in the urine of different species of animals but even pituitary grafts from pregnant cows (Bacon, 1930) and pregnant sows

(Wolfe, 1931) have failed to show an increase in potency. It is likewise peculiar that pituitary glands from dogs had little or no action when grafted into monkeys (Allen, 1928). These variations although difficult to understand serve as a stimulus to further research.

SUMMARY

1. The amount of anterior pituitary sex hormones in the blood and urine of several types of rat donors was tested by injecting immature female rats.

2. Positive results, as represented by enlargement of the ovaries and uterus and signs of oestrus, were obtained with castrated male and castrated female sera.

3. The hormone was not found in serum of normal males, normal females, multiparous or pregnant females.

4. Tests with urine collected from normal males, castrated males and females and pregnant rats were all negative.

5. Extracts made from urine were likewise negative.

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METABOLISM DURING GROWTH IN A COMMON PIGEON

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The effects of age upon the basal metabolism, particularly in the period from birth or hatching to maturity, are known in very few animals. Fairly adequate data for the human were obtained by DuBois (1916), Benedict (1919) and Benedict and Talbot (1921). Deighton (1924) and Wood (1926) obtained the essential facts for the pig; Mitchell, Card and Haines (1927) supplied important data for the fowl; and Brody and Ragsdale (1930) provided significant details for the calf. It is remarkable that the newborn of all these four species have an initial low metabolism which rather quickly rises to a maximum and then declines during maturity and adult life. It is found that this same situation reappears in the results of the present study. A few measurements have been made on the metabolism of nestling birds, including pigeons. Pembrey (1897) found that, though the chick is homothermic at hatching, the young pigeon begins to give evidence of this property only at 7 or 8 days after hatching, and only at 15 days was its heat regulation as well developed as that of the newly hatched chick. Leichtentritt (1919) and Plaut (1921) found a relatively low metabolism in young nestlings which Groebbels (1927) failed to confirm in pigeons, probably because of inadequate data and of failure to recognize the very short duration of this low metabolism in this species.

In the present study the metabolism during six early stages of growth, besides an early (107 days) and late (164 days) phase of "adolescence" and a still later stage representing adult life, was measured in a small and well-marked race of common pigeons known as tipplers. On these nine age-groups 130 measurements were made. The earliest stage used was 3 days after hatching, which represents 21 days from the beginning of embryonic development. Because of other and current studies on these birds it is desirable here to calculate age from the beginning of development, not from hatching. Those who wish to think of age as dating from hatching may do so by subtracting 18 days from the values stated in this paper. At 21 days, and for more than 10 days thereafter, the rate of growth in the pigeon is extraordinarily rapid (see table 1), and the metabolism measurements made at these two earliest stages are therefore of exceptional interest. Other

measures were made at 41-day and 43½-day stages when growth is still rapid but distinctly less so than in the preceding periods.

All these very early growth stages (to 50 days) were measured on non-fasting birds (excepting 3 birds aged 43 days, as noted later), because it was further sought (successfully on 10 birds) to measure the same individuals at several successive growth stages. No special preliminary preparation was used in the case of these youngest birds; they were taken directly from their nests to the metabolism chamber; they remained in the metabolism chamber at 30° for approximately 6 hours; they had been gorged with food shortly before beginning the test, and at its end they usually had much food in their crops; the weight of the crop contents was accurately estimated, and this quantity subtracted from the total weight of the bird. All birds more than 50 days old, however, were fasted for 24 hours and subjected to those several conditions of preparation and measurement which Benedict and Riddle (1929) have earlier described as necessary to basal measurements in the pigeon. These conditions, we would like to emphasize, include a 24-hour fast (begun, in birds weighing 200 to 260 grams, with 8 grams' grain and 8 grams' water in the bird's crop) spent in a large glass cage kept at 30°C.; with measurements made at 30°C., at night, in completely darkened chambers; and with activity recorded by kymograph. Since the sex of young pigeons can not be recognized no attempt has been made to analyze our data on the basis of sex. We have, however, assured ourselves that the two sexes are fairly distributed in all age-groups.

Somewhat arbitrarily, yet almost necessarily, we selected 30°C. as the environmental temperature at which to make all measurements. This temperature seemed the lowest at which we could keep the youngest birds comfortable in the metabolism chamber—even with a protecting bed and covering of absorbent cotton. Birds of the 29-day stage were provided with this bed, but with less covering. Most, though not all, of these very young birds seemed comfortable when removed from the metabolism chamber; the rectal temperatures then obtained, and the relative quiet during measurement, also indicated that the covering supplied to these birds enabled them to remain essentially normal and comfortable in the 30° environment. We do not suppose, however, that the temperature used was equally agreeable and suitable to these many stages of a rapidly changing organism. Indeed, we know that the birds of the 21-day stage, while normally covered in their nests by their parents, are usually given an environmental temperature in excess of 35°C. (their covering there being the feathers and skin of the parent); also that the "critical" temperature of the adult pigeon is approximately 30°C. and measurements made on them at 33° or 35° give higher metabolism values than are obtained at 30°C. (unpublished data). Surface was calculated from the formula: $S = 10 \times W^{\frac{2}{3}}$. Here S = square centimeter, and W = body weight in grams.

EXPERIMENTAL RESULTS. A summary of results is given in table 1. The very youngest stage (21 days, 3 days after hatching), though not fasted and in extremely rapid growth, has a metabolism not much higher than that of the fasting adolescent bird (107 to 164 days). Though crammed with food this youngest stage gives a respiratory quotient which indicates that little or no carbohydrate is being burned; and this corresponds well with the observation that at this stage these young are fed almost exclusively on "crop-milk"—a secretion containing much fat, some protein and no carbohydrate. On the other hand, the other and next stage of extremely rapid growth (29 days) is indicated as having the highest metabolism (1284

TABLE 1

Summary of data from measurements of the respiratory metabolism during growth in a common pigeon (tippler)

All measurements made during March–June, at 30°C.

AGE (FROM BEGINNING OF DEVELOPMENT)	BODY WEIGHT (END OF TEST)	CONDITION	R.Q. (AVERAGE)	CALORIES PER SQUARE METER PER 24 HOURS	NUMBER OF TESTS
days	*grams*				
21	29.6*	Not fasting	0.768	773	13*
29	127.3	Not fasting	0.991	1,284	24
41	231.1	Not fasting	0.987	1,125	12
43.5	247.6	Not fasting	0.980	1,050	12
43.3	221.8	Fasting 24 hours	0.745	922	3
56	211.8	Fasting 24 hours	0.726	727	16
80	237.0	Fasting 24 hours	0.731	719	14
107	236.8	Fasting 24 hours	0.707	737	8
164	247.7	Fasting 24 hours	0.703	632	9
439	254.3	Fasting 24 hours	0.760	638	15

* In this group 13 pairs, or 26 birds, were used since a single bird was too small for satisfactory measurement.

calories per square meter per 24 hours) attained during the entire life cycle, and at this and the two next following stages chiefly carbohydrate is burned—a fact shown by respiratory quotients of about 0.99. At the age of 41 and 43½ days the total metabolism is definitely reduced below the 29-day value. It is at 30 to 45 days that the plumage becomes a very effective agent for the conservation of heat, and this may be concerned in the marked and progressive decrease in heat production observed at this period. When 30 days old the young are not continuously covered by their parents, except in cold weather; at 40 days (22 after hatching) the young have already deserted the nest and, though they seek and require other means of shelter, they are no longer brooded by the parents.

The values found for age-groups which were fasted before measurement indicate, in general, a small progressive decline in the metabolism from

the 56-day stage to the 435-day stage; between 56 and 107 days, however, such a decline is not indicated by our data. We have attempted a measure of the extent to which the very high metabolism at the 43-day stage is influenced by the non-fasting condition of this group. Three such birds were measured first in the non-fasting condition and again 24 hours later after fasting under the precise conditions used with the older groups of birds. The results of this special test must be described although they are partly

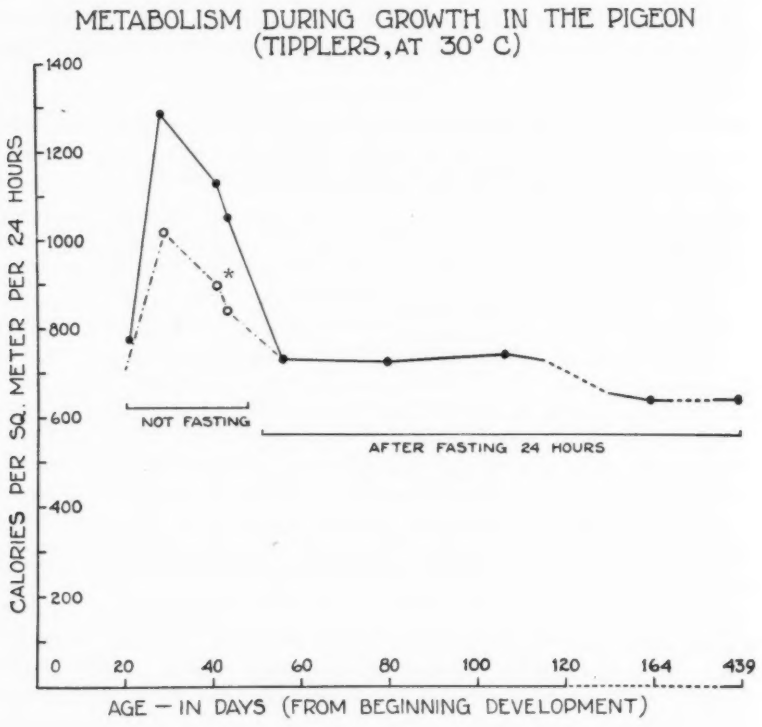

Fig. 1

given in table 1 and in figure 1. The average value obtained from these three fasting birds was 922 calories (separately represented by a * on the curve); their own non-fasting value was 1160 calories. The latter value is therefore 125.8 per cent of the fasting or basal value. If we may suppose that the measured non-fasting values for all birds of the 43-day, 41-day and 29-day stages bear a similar relation to their basal values we obtain the points (open circles) connected by the broken line on figure 1. This

broken line is further extended to a point somewhat below the measured value of the 21-day stage for the reason that the birds of that stage, though burning only fat and protein, were certainly digesting and absorbing food and therefore not in a truly "basal" condition. That portion of the curve which is represented by open circles and dotted lines, together with its continuation in the curve obtained from fasting birds of older stages, best presents the "basal" metabolism of the tippler pigeon during growth when measured at all ages at 30°C.

Details of measurements made at the two youngest stages of growth are of special interest and these are given in table 2. The birds taken for a particular growth stage were not of precisely uniform age, and they varied markedly in weight; an inspection of these differences will supply further evidence for the validity of the first and most irregular part of the growth curve presented in figure 1. Within each age-group it will be noted that smaller size and younger age tend to be associated with a lower metabolism; greater body weight and more advanced age—up to 30 or 32 days—tend to have the higher metabolism. In this connection it should be noted that within very wide limits the actual rate of growth in all pigeons and doves depends upon the volume of the food supply; and that where there is but one young in a nest its growth, during the first 10 days after hatching, may nearly equal that of two squabs in the same nest. Thus no. 24 of table 2 was the sole occupant of a nest; its weight (189 g.) at 29 days (11 days after hatching) was fully twice that of no. 4 (92 g.)—a squab of the same age forced to share with a nestmate the food supplied by its parents—and the metabolism of the former and rapidly growing bird exceeds that of the latter by 20 per cent. Many similar instances of this relationship may be found in this table when one compares two birds of the same age but of very unequal size. We here find a more rapid growth reflected in a higher metabolism. The birds thus compared are of course quite unequal in size; the metabolism values of this paper are, for these intra-specific comparisons, all calculated in terms of heat per square meter of surface.

The rate of growth, in terms of percentage weight increment at the various ages, should be considered. For this purpose we shall use some data of Riddle, Charles and Cauthen (1932) which includes the weights of 15 tipplers from hatching to 105 days. Those birds were never fasted and so present a fair picture of the normal growth of birds of this race. The weights, in grams, for several stages are: Hatching (17 to 18 days) 11.3; 21 days, 30; 28.7 days, 130; 41 days, 230; 43.5 days, 237 grams. A comparison of these values with those obtained (table 1) from the (non-fasting) birds used in the present metabolism measurements makes it clear that the present studies were made on birds growing at the usual or normal rate; also that the repeated measurement of 10 of these birds was without notable effect on their rate of growth. In the other study of the growth rate in

TABLE 2

Individual measurements made on pairs of young at the 21-day stage—20–22 days after beginning of development

Pairs arranged in order of body weight. Next older stage listed below

PAIR (OR NUMBER)	BODY WEIGHT PER BIRD	AGE	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER 24 HOURS	EXACT CHAMBER TEMPERATURE
	grams	*days*			*°C.*
1	13.5	21	0.67	624	29.9
2	21.5	21	0.74	674	29.9
3	23.7	20	0.78	675	30.0
4	24.0	22	0.69	898	30.0
5	27.0	21	0.74	778	30.1
6	27.3	21	0.74	773	29.9
7	28.3	21	0.85	701	29.9
8	30.0	21	0.86	742	30.0
9	32.0	21	0.68	914	30.1
10	33.0	22	0.82	828	30.0
11	37.0	21	0.74	875	29.9
12	43.5	22	0.79	846	30.0
13	44.0	22	0.89	714	29.8
Average....	29.6	21	0.768	772.5	29.96
Individual birds, 10–14 days after hatching (29-day stage)					
1	88.5	28	0.96	1,148	30.0
2	89	27	1.00	1,261	30.0
3	91.5	27	0.97	1,142	30.0
4	92	29	0.98	1,066	30.2
5	107.0	30	0.86	951	30.2
6	110.5	29	1.08	1,283	30.1
7	117.5	28	0.84	1,095	30.0
8	119	29	1.10	1,145	30.1
9	119	28	1.06	1,312	29.9
10	121	28	0.92	1,150	30.0
11	122.5	29		1,324	30.0
12	124	27	0.92	1,362	29.9
13	127.5	29	0.87	1,234	29.9
14	130.5	28	1.02	1,410	30.1
15	131.5	29	1.04	1,366	30.0
16	134.5	32	1.03	1,231	30.2
17	135.5	27	0.88	1,451	30.1
18	136	28	0.95	1,266	30.0
19	136.5	28	1.05	1,473	30.0
20	149	30	1.11	1,373	30.1
21	159	29	1.09	1,640	30.1
22	162.5	30	1.04	1,408	30.0
23	167	32	1.00	1,435	30.2
24	189	29	1.03	1,285	30.2
Average....	127.5	28.75	0.991	1,283.8	30.05

tipplers weighings were made at 3-day intervals and this permits the following statement on the approximate percentages of growth increment per day at the several stages concerned in the present study: At 21 days, 38 per cent; at 29 days, 14 per cent; at 41 days, 2 per cent; at 43.5 days, 1 per cent. After 45 to 50 days the young are no longer fed by their parents, and our practice is to remove the young when 50 days old. For a few days thereafter they lose weight slightly, and this short period is followed by very slow growth during a few succeeding months. The preceding statement will indicate that, with the important exception of the earliest 21-day stage, the metabolism curve essentially parallels a curve which we might construct for growth increment in this race of pigeons.

Since the present study is especially concerned with extremely early life-stages, and the metabolism of the youngest stage examined bears a peculiar relation to all values obtained later, it is particularly important to consider the adequacy and meaning of the measurements made on our birds 3 days after hatching. The details supplied in table 2 leave no doubt that the value obtained for this stage is approximately correct. These extremely young birds supplied evidence that they already have some capacity to maintain their body temperature considerably above that of their environment. The rectal temperatures of 4 such birds, after remaining for 6–8 hours at 30° in a chamber ventilated at the rate of approximately 2 liters per minute, had body temperatures ranging from 39.4 to 40.0°C.; another group of 4 similarly kept at 36° showed a range of 40.0 to 41.3°. Nine birds of this same age, while covered in the nest by a parent, had an average temperature of 39.2°C. Birds of this stage, given such protection from heat loss as was supplied to these birds, are therefore almost or quite capable of maintaining their normal body temperature when the temperature of the environment is 10°C. lower than their own. These data nevertheless show that when birds of this stage are long held in the chamber at 30° they have a body temperature nearly 2°C. lower than that of the adults, and that their relatively low metabolism was obtained from tissues at a temperature distinctly lower than in all subsequent measurements. When kept in an environment held constantly at 36° these birds seem, however, to maintain body temperature almost identical with that of the adult. Only 8 days later (29-day stage) the average body temperature (night) of the 24 birds studied (at 30°) was 40.4° (1 at 35° was 41.5°), while that of the adults was very slightly less than 41°C.

It can not be assumed that an environmental temperature of 30° is of the same significance to thermolysis in a bird 3 or 11 days after hatching as in the warm-blooded adult pigeon. The "critical" temperature for the adult is approximately 30°C.; but, as suggested by Pembrey's studies, it does not follow that a truly comparable metabolism of unfeathered, partially cold-blooded young of 3 or 11 days is to be obtained at this temperature. Four

measurements (not tabulated) were made in order to learn the relation between the values obtained at 30° and values obtainable at still higher temperatures (35° or 36°C.) on these particular stages. A pair of 20-day young (weight, 14 and 27 g.) and another pair of 20.5-day young (16 and 21.5 g.), at 36°, produced 745 and 710 calories per square meter per 24 hours; from a pair of 25-day young (65 and 80 g.), at 36°, we obtained 1137 calories, and from a 30-day young (107 g.), measured at 35°, 1330 calories. The values obtained from the age-groups thus tested invariably stand in the same relation to each other as when measurements are made at 30°; the heat production at the 25-day and 30-day stages is somewhat increased at the higher temperature; in the 20-day birds, when age and size are fully considered, the metabolism is perhaps 8 per cent higher than that to be expected at 30°. The general form of the curve constructed from 30° values is not changed when the partially cold-blooded stages are measured at a higher environmental temperature at which their cell temperature is made equivalent to that of the adult; at this higher temperature the youngest stage (20-day) again shows a low metabolism, and the 29-day stage again has the highest metabolism of the life cycle.

That a pigeon, while largely (21-day stage) or partially (29-day stage) a cold-blooded animal, should duplicate both the initial low and the succeeding high metabolism found in the warm-blooded young chick is of much interest. That the two cases in birds already find a complete parallel in the three mammals (human, pig, calf) hitherto studied makes it evident that something very important in the physiology of development is involved. The fact that the relatively low initial metabolism occurs in the pigeon when the animal is in phenomenally rapid growth, and the further fact that the maximum metabolism occurs when growth rate (though very rapid) has passed its maximum, demonstrate the extreme complexity of the problem. We do not undertake a discussion of this problem here; we emphasize the now evident fact that for these two phases of metabolic change during early life there is at present no adequate explanation.

SUMMARY

The total metabolism, at 30°C., of a race of common pigeons (tipplers) has been measured at four early stages of rapid growth; basal values were obtained on one of those early stages, at three later periods of slower growth, at early and late adolescence, and after full sexual maturity. The results are based upon 130 measurements.

Three days after hatching (21-day stage), when non-fasting but burning fat only, the bird is in a stage of most rapid growth (38 per cent per day) but it then has a total metabolism only 20 per cent higher than the basal value of the young adult. Eight days later the total non-fasting metabolism (R.Q. of 0.99) is about 100 per cent higher (1284 calories per square

meter per 24 hours) than the basal value at late adolescence (632 calories). This extraordinarily high metabolism coincides with a period of very rapid growth (14 per cent per day) in this animal.

When 41 and 43 days old (23 and 25 days after hatching) the total metabolism and the rate of growth are in rapid decline. At this time measurements made on both fasting and non-fasting birds show that the metabolism is markedly greater (922 calories) than in birds of the 56-day (727 calories) and still older stages.

In general, the basal heat production progressively declines from the 43-day—and probably from the 28-day stage—into adult life; from the twenty-eighth day (10 days after hatching) the decline in metabolism parallels a decline in growth rate.

The curve describing the changes of metabolism with age in the pigeon —an animal which is partially cold-blooded at an early and significant period —is strikingly similar to that already known for man, pig, calf, and fowl. A low metabolism immediately after birth or hatching, this soon followed by the highest metabolism of the life cycle, and the invariable occurrence of both of these things in all of the five species hitherto studied, are challenging facts for which no adequate explanation is now at hand.

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THE BASAL METABOLISM OF THE MOURNING DOVE AND SOME OF ITS HYBRIDS

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The mourning dove (*Zenaidura macroura*) is the only truly feral species of Columbae (pigeons) which is native to New York. Here it is found in considerable numbers during spring and summer. Nearly but not quite all non-captive individuals migrate southward, to borders of the Gulf of Mexico, in autumn; they return to this locality in April. It is a truly migratory species—avoiding cold weather—and is thus in sharp contrast with the common pigeons and ring doves which we are studying intensively. In these latter species we are observing distinct seasonal differences in the basal metabolism, and also differences in the extent to which the environmental temperature influences the metabolism at the various seasons. The migratory mourning dove, when held captive and measured at various temperatures at various seasons, should therefore provide information of special interest. We have also sought to learn something of the extent to which age, sex and hybridity modify the metabolism in this feral species.

Our stock of birds was formed from a few individuals captured as adults or young near the laboratory; the offspring of these birds are the individuals used in this study. During 3 years we have made 150 measurements upon 39 different individuals. On 15 hybrids 30 measurements were made. The hybrids to be considered here are those derived from mating the mourning dove with a not very dissimilar species from South America (*Zenaida vinaceo-rufa*). The two parent forms are at present classed in different genera, but their full fertility without marked disturbance of the sex ratio may be taken as evidence that they might well be placed in the same genus. The Zenaidura-Zenaida hybrids, hereafter to be called Z-Zn hybrids, are fertile, and most of the individuals used in these tests were derived from generations later than F_1. Curiously enough the pure Zenaida, a tropical species, is able to produce fertile eggs during all months of the year when given the protection from extreme cold which our glass colony houses afford.

All measurements were made under the standard conditions for pigeons

as earlier described by Benedict and Riddle (1929). These include: Previous fasting for 24 hours (starting this fast, in these small doves, with 5 grams of food plus 5 cc. of water in the crop) during which time the birds are kept in a large glass cage at constant temperature (and the *same* temperature as that to be used in the metabolism measurement); making all measurements at night, with kymograph records of activity, and in completely darkened chambers. Measurements were never started until at least one hour after placing the birds in the darkened chambers, and then only if quiet had been obtained. Closely corresponding values derived from two periods of complete or essential quiet constitute a measurement. Sick or unhealthy birds are excluded. The R.Q. was obtained in 74 of the 150 measurements on mourning doves; its range was 0.67 to 0.76 and its average, 0.718. Similarly, 11 quotients on the Z-Zn hybrids average 0.703. The length of the fasting period (always 24 hours) used with these birds was therefore entirely adequate to obtain fat quotients; perhaps such quotients could have been obtained 4 or 5 hours earlier. In that case a slightly higher metabolism would have been obtained, and the differences we have found between the metabolism of this dove and other domesticated doves and pigeons would be slightly increased.

CONSIDERATION OF DATA. The data for the different seasons, temperatures, sexes, ages and types of birds are summarized in table 1.

Season. In this region non-captive mourning doves lay and fertilize eggs only from April to the end of July; captive birds may begin breeding slightly earlier and continue to the end of August. Soon after the end of the breeding season, but persisting only through the autumn in captive birds, the testes of this species undergo a seasonal involution which is so extensive as to suggest effective functional castration. The time and extent of this involution of the testes are indicated by the following weights of testes of adult doves (both captive and wild) at various months: The average for 6 in May was 514 mgm.; of 5 in August, 435 mgm.; of 4 in September, 87 mgm.; of 2 in October, 18 mgm.; of 5 in December, 396 mgm. Taber (1928) measured the food consumption of this species during the breeding season and in September, and drew the conclusion that "it seems probable that there is a seasonal variation, because the daily food consumption of 13 mourning doves trapped from April 2 to August 1 averaged 11.7 per cent of their body weights, while the daily food consumption of the 9 trapped from September 6 to October 6 averaged 20.4 per cent."

Though the ovary becomes smaller and ovulation ceases before September this gland does not suffer a diminution comparable with that observed in the testis. In the Z-Zn hybrids the gonads are reduced in winter, but much less so than in the pure species and eggs are usually produced in all months except October–January. How then is the basal metabolism related to these seasonal differences in these animals? In our survey of this

TABLE 1

Summary of data on basal metabolism of mourning doves (top) and hybrids

AGE CLASS	SEASON	CHAMBER TEMPERATURE	SEX	AGE	BODY WEIGHT	NUMBER OF TESTS	CALORIES PER KILO PER 24 HOURS
		°C.		*months*	*grams*		
Immature	Winter	30	♂	9.1	119	5	131.9
			♀	7.1	137	3	119.0
Mature	Breeding	30	♂	26.6	122	8	123.1
			♀	27.8	114	7	117.5
Mature	December	22	♂	46.3	142	6	185.1
			♀	33.9	124	6	165.5
Mature	Breeding	20	♂	27.1	119	19	179.0
			♀	23.8	118	20	175.6
Mature	September	20	♂	34.2	125	5	198.2
			♀	26.1	117	6	195.8
Mature	November	20	♂	29.0	131	10	179.4
			♀	23.8	127	11	168.8
Mature	Winter	20	♂	34.4	137	11	172.3
			♀	29.2	131	11	180.8
Immature	Winter	20	♂	7.4	130	7	172.9
			♀	7.6	116	5	177.6
			?	7.0	115	2	187.3
Immature	Winter	15	♂	7.2	119	5	217.0
			♀	7.1	125	3	200.6
Hybrids—Mourning dove X *Zenaida vinaceo-rufa*							
Mature	Winter	22	♂	54.4	108	4	174.6
			♀	39.5	103	4	170.4
Mature	Winter	20	♂	34.6	102	4	192.6
			♀	36.8	105	3	187.4
Mature	Breeding	20	♂	39.4	103	5	184.8
			♀	38.4	98	4	166.4
Immature	Winter	20	?	7.9	102	4	188.5
			♀	6.9	102	2	167.0

point it is necessary to observe that in our work (mostly unpublished) on ring doves and pigeons we find the highest metabolism in the autumn period (September–November), and usually the lowest in summer (June–August), with winter values (December–February) not very much higher than those of summer probably because the colony houses are enclosed and partly heated during the winter (not in autumn).

Our examination of the effects of season on metabolism in the present study is limited essentially to measurements made on mature birds at 20°C. The 39 mourning doves measured (April 28 to August 6) during the breeding season give a mean sex value of 177 calories per kilo per 24 hours, and this is essentially the same as that obtained for November (174) and for winter (177); it is also apparently the same as the December value (175) obtained at 22°, but probably it is 3 to 5 per cent lower than the December value when a derivable temperature correction is made. All these values, however, differ notably from that obtained in September (197) the latter being 11.1 per cent above the summer metabolism. Thus the highest value is found at a time when the testes are being actively reduced, but the very fact that the testes are being resorbed makes it entirely probable that they are then contributing some sex hormone to the body; also, this would seem to be the time when the thyroids should be actively enlarging in response to colder weather—if the thyroids of these birds enlarge in cold weather as do the thyroids of ring doves and common pigeons (Riddle and Fisher, 1925).

A tentative explanation of these unusual and unexpected seasonal values may be offered. It is conceivable that the "breeding season" values reflect two opposed tendencies: First, a tendency to a lowered summer metabolism (as in other doves and pigeons) accompanying reduced thyroid activity; second, a failure of the summer metabolism to become actually lower because of a stimulating action of the gonad hormones—whose presence *in this species* is probably essentially limited to this breeding period. We may likewise suppose that the actual level of the metabolism in November is the resultant of two things—the loss of gonad hormones (tending to lower it), but a gain of thyroid activity (in response to cold) which increases it. However, the fact that the "winter" value is no higher than that for the breeding season, does not well fit this interpretation since, as noted above, the testes have then recovered much of their former size and presumably much of their endocrine function. Again, the high metabolism in September may be partially or wholly a result of less effective feathering coincident with a possible predominance of molting at this period, though we lack evidence that this is the molting period for this species. Even this very questionable disposition of the high metabolism in September would leave wholly unexplained the equivalence of the metabolism in summer, November and winter.

If the explanation suggested above is erroneous or inadequate it would seem necessary to conclude that in this migratory, cold-avoiding species the thyroids do not respond to cold by increased function as they do in non-migratory doves and pigeons. In that case our results suggest the probability of a causal relationship between thyroids which do not make the normal response to cold and the presence of the migratory instinct in birds possessing such thyroids. Special evidence on this point is found in the fact, first observed by Riddle and Fisher (1925), that the thyroids of non-migratory ring doves and common pigeons undergo marked enlargement and increased function during autumn and winter, and by Haecker's (1926) later demonstration of the same thing in the non-migratory European crow.

Environmental temperature. Immature mourning doves were measured in winter at 15°–20°–30°C. Neglecting here a small sex difference (the sex of 2 birds being unknown) the tabulated values indicate that the metabolism at 20° has been reduced by 16.2 per cent below the 15° value; at 30° their metabolism, based on the means for the sexes, is reduced by 29.8 per cent below the value found at 20°. Thus a rise of temperature from 15° to 20° lowers the metabolism by 3.23 per cent per degree, and between 20° and 30° the rise in temperature lowers this metabolism, on the average, by 2.98 per cent per degree. A similar comparison for the mature doves can be made only for 20°–30°, and in the "breeding season." Here the metabolism at 30° indicates a loss of 3.2 per cent per degree from the value found at 20°C.

It should be noted that these percentage changes in the metabolism per unit of change in the environmental temperature seem relatively greater in the mourning dove than those found in ring doves and pigeons. Riddle, Christman and Benedict (1930) found that in ring doves the two sexes gave a different response to temperature change, but that between 20° and 30° an average was 2.61–2.99 per cent in males, and 2.03–2.25 per cent per degree in females; between 15° and 20° this value was 2.33 in males, 2.02 in females. Our own data (1932) on a race of common pigeons (tipplers) show that, though values obtained from calculations of this sort depend essentially upon season, the metabolism change for measurements made on those birds at 30° and 20° lies between 0.3 and 1.0 per cent per degree; between 15° and 20° the extent of the influence upon the metabolism ranges between 1.3 and 3.4 per cent per degree. These values for common pigeons are the mean values for the sexes. Thus almost all of the several available comparisons indicate that the metabolism of mourning doves, though apparently less modified by prolonged seasonal temperature change, is more increased by a temporary lowering of the environmental temperature (for purposes of measurement) than is that of ring doves and common pigeons. In other words, the migratory species studied here must

do more work—must produce relatively more heat—to maintain itself in a cold climate than is required in two somewhat related non-migratory species. Since the mourning dove is only slightly smaller than the ring dove we here again meet a fact that may be intimately, perhaps causally, related to the migrant character of this species.

Age. Measurements made at 20° on mourning doves during the winter, when the gonads of immature and mature birds are relatively inactive and when body size in the immature is nearly equal to that of the adult, indicate that birds 6 to 8 months old have nearly or quite the same metabolism as birds aged 20 to 50 months. This "winter" metabolism of the immature birds is also entirely similar to the metabolism of mature birds in November and in the breeding season, but is definitely lower than that of birds 20 to 50 months old measured in September. The metabolism of eight immature birds measured at 30° in winter seems only slightly higher (4.3 per cent average) than that of fifteen mature birds measured at this temperature during the breeding season. A few measurements made on Z-Zn hybrids 6–9 months old at 20° during winter indicate no superiority of their metabolism over that of either of the two groups of mature birds measured at 20° in winter. These data make it entirely probable that, between 6–50 months, age is a negligible factor in the metabolism of mourning doves and their hybrids.

Sex. Within the several groups studied there is a tendency for the males to be slightly older and larger than the females. Much experience has taught us that the relation of the sex factor to metabolism in species and races of doves and pigeons can not be adequately determined by a few scores of measurements; nor yet by many hundreds of measurements made at a single temperature which may affect the metabolism of the two sexes differentially. In this migratory, cold-avoiding species we note that the metabolism of males is not obviously depressed more than is that of females by measurement at an environmental temperature of 30°C. The present data were obtained at four different environmental temperatures and seem to give significant indications concerning the sex factor in the metabolism of mourning doves, and probably also in the hybrids. At all of the four temperatures used the male mourning doves are indicated as having a basal metabolism higher than that of the females; in 7 of the 9 groups measured the males show the higher value; the means obtained from the 9 group values indicate that the males have a metabolism 3.8 per cent in excess of that of the female when the heat production values are calculated on the basis of body weight, and when calculated in units of body surface this excess is 4.6 per cent.

Even the fewer data concerning sex in the Z-Zn hybrids are not negligible since, in the 3 groups studied, they afford consistent evidence that the metabolism of the males at 20° to 22° is higher than that of the females.

The means derived from the three available groups indicate that the males have a metabolism 5.3 per cent higher on the basis of weight, and 6.3 per cent higher per unit of body surface. These data have an added interest from the fact that they seem to be the first specific (or generic) hybrids upon which measurements of the respiratory metabolism have been made.

Species. The metabolism of the mourning dove may be compared with that of ring doves. For mature individuals of 16 races of the latter species, measured at all seasons at 20°C. (a few at 22°), a mean value of 155 (males) and 150 (females) calories per kilo per 24 hours (811 and 774 per square meter per 24 hours) was found by Riddle, Christman and Benedict (1930). These values are somewhat lower than those here obtained for the mourning doves at 20° to 22° (table 1). Five groups (all seasons) of mature mourning doves give mean values of 183 and 177 calories per kilo per 24 hours, or 923 and 885 per square meter per 24 hours, in males and females respectively. Some wholly comparable data for a small race of common pigeons (tipplers) gave 61 males a value of 105 calories per kilo per 24 hours, and 673 calories per square meter per 24 hours; from 70 females we obtained 108 calories per kilo per 24 hours, and 687 per square meter per 24 hours. Other races of normal common pigeons do not greatly exceed this value. It is thus shown that the feral species has a metabolism higher than that of domesticated doves and pigeons. We have evidence that the true normal rectal temperature of the mourning dove is no higher than that of ring doves or common pigeons. The metabolism of the wild or Norway rat was likewise shown by Benedict and Petrik (1930) to exceed that of the domesticated albino rat.

The Z-Zn hybrids have a body weight about 20 per cent less than that of mourning doves and probably only about equal to that of the pure Zenaida ancestry—but unfortunately only a single female of the latter species was available for study. The metabolism of the hybrids is equivalent to that of the mourning doves when calculated on the basis of weight (184 and 175 calories in males and females), but about 7 per cent less when calculated on a basis of surface area (871 and 813 per square meter per 24 hours). Estimates of surface area in these several calculations assume that the area varies in proportion to the two-thirds power of the body weight; K = 10.0.

SUMMARY

Individuals of a feral, cold-avoiding, migratory species of dove, when reared in captivity, have been found to have a higher basal metabolism, measured at 15°, 20° and 30°C., than that found in related non-migratory domesticated doves and pigeons. At 20°, and measured at all seasons, the mean values found for the two sexes in these several species are: 904

(mourning dove), 792 (ring dove), and 680 (tippler pigeon) calories per square meter per 24 hours.

Seasonal changes in the metabolism of this species are especially difficult to interpret because possible responses to hot and cold weather and an extraordinary autumnal involution of the gonads, apparently the equivalent of functional castration, are involved. The highest metabolism is found in September; lower and quite interchangeable values are found during the breeding season, November and winter.

It is possible that the thyroids of this species do not respond to cold weather by increased activity as do those of non-migratory forms and that this is significantly or causally related to the migratory instinct.

This migratory, cold-avoiding species must do more work—must produce relatively more heat—to maintain itself at lower temperatures than is required in either of two related non-migratory species of dove already studied.

Age is here a negligible factor in the rate of heat production of birds whose ages range between 6 and 50 months.

The metabolism of male mourning doves, as measured at all temperatures and during all seasons, was higher (173 per kilo, and 873 calories per square meter, per 24 hours) than that of females (167 and 829 calories). Per unit of weight this excess is 3.8 per cent, and per unit of surface it is 4.6 per cent.

Thirty measurements made at 20° and 22° on hybrids between the mourning dove and the Zenaida dove indicate a metabolism (179 calories per kilo per 24 hours) that is nearly the same as was found in the mourning dove (180 calories), that is equal in younger and older birds, and that is higher by 5.3 per cent (weight) or 6.3 per cent (surface) in males than in females.

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THE PHYSIOLOGIC ACTION OF DEHYDROCHOLIC ACID

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Dehydrocholic acid, according to Neubauer, caused a two- to fivefold increase in bile secretion on intravenous injection in rabbits and in patients with biliary fistulae. On the basis of other work on cholic and desoxycholic acid, he stated that the cholagogic bile acids increase the bile secretion pressure.

Neubauer reported further that dehydrocholic acid is less hemolytic (human, rabbit erythrocytes) than desoxycholic acid, that it is $\frac{1}{2}^{1}_{5}$ as toxic to the excised frog heart as desoxycholic acid, and that it has only about $\frac{1}{8}$ the toxicity of desoxycholic acid for guinea pigs. This was confirmed by Gillert.

Neubauer injected into one dog weighing 8.8 kilograms on different days sodium dehydrocholate in the following amounts: 0.6 1.55, 3.2, 3.8, grams, that is, from 0.068 to 0.43 gram per kilogram of body weight, in from 6 to 32 per cent solutions, without toxic effects. On injection of 0.51 gram per kilogram intravenously vomiting and diarrhea were observed. The intravenous injection of 2 grams of sodium dehydrocholate in 20 per cent solution in patients was without toxic effects.

In beginning our work solutions of sodium dehydrocholate[1] and sodium choleate were injected into frogs (Rana pipiens) weighing from 40 to 60 grams. Some injections were made into the anterior lymph sac and some into the posterior lymph sac. The salts were prepared by dissolving the acids in warm alcohol and neutralizing with N/10 sodium hydroxide, using phenolphthalien as the indicator. The solutions were then evaporated to dryness and the salt taken up in distilled water.

In tests on 36 frogs with sodium dehydrocholate (0.2 to 5.0 millimols per kilogram) and 14 frogs with sodium choleate, the sodium dehydrocholate proved to be about $\frac{1}{5}$ as toxic as the latter salt.

On intravenous injection of 20 per cent solution of sodium dehydrocholate in seven dogs, defecation, vomiting, hyperexcitability and death were observed following doses ranging from 0.024 to 0.72 gram per kilogram body weight. Similar reactions were produced by sodium glyco-

[1] The dehydrocholic acid (Decholin) used in all these experiments was obtained from the Riedel-de Haen Company.

cholate in smaller doses. Hypodermic injections of sodium dehydrocholate in concentrations up to 20 per cent do not induce local necrosis as does sodium glycocholate.

In all the following experiments the dogs were under barbital anesthesia—300 mgm. per kilogram per os. In all experiments whether determining bile flow or bile pressure the common bile duct was cannulated and the cystic duct ligated. All injections were made into the femoral vein. The dose was arbitrarily taken as 10 cc. of 20 per cent solution or 2 grams since it is the calculated human dose. The solutions were always warmed

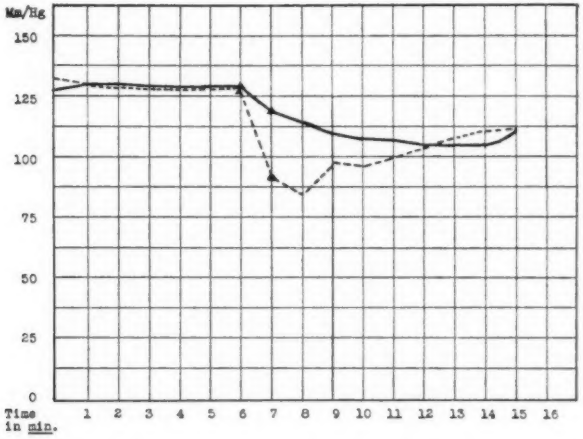

Fig. 1. The effects of intravenous injection of sodium dehydrocholate and sodium glycocholate on blood pressure in barbitalized dogs.

———— Average blood pressure of 18 dogs following injection of 2 grams of sodium dehydrocholate.

---------- Average blood pressure of 11 dogs following injection of 2 grams of sodium glycocholate.

▲—▲ Injection.

to body temperature and the injections made at a constant rate in about one minute.

Figure 1 gives the average fall in blood pressure in a large number of dogs following the intravenous injection of 2 grams of sodium dehydrocholate and the same dose of sodium glycocholate (Merck). The limits of fall were from 4 to 80 mm. of mercury for sodium dehydrocholate and from 24 to 104 mm. of sodium glycocholate. It is readily seen that sodium dehydrocholate has less effect on blood pressure than sodium glycocholate.

There was no change in the average respiratory rate of 19 dogs following injection of 2 grams of sodium dehydrocholate. However, nine dogs

showed an increase in rate and ten dogs a decrease. With similar amounts of sodium glycocholate there was an average decrease in respiratory rate of 18.70 per cent. Eight dogs showed a decrease whereas three dogs showed no change.

TABLE 1

Amounts of bile secured in 15 minute intervals

DOG	AVERAGE BASAL	AVERAGE FOR 2½ HRS. AFTER INJECTION OF Na DEHYDROCHOLATE
	cc.	*cc.*
344	0.85	7.21
345	0.44	6.38
346	0.76	4.96
347	1.30	6.30
348	0.46	4.30
350	0.69	6.05
351	0.33	6.26

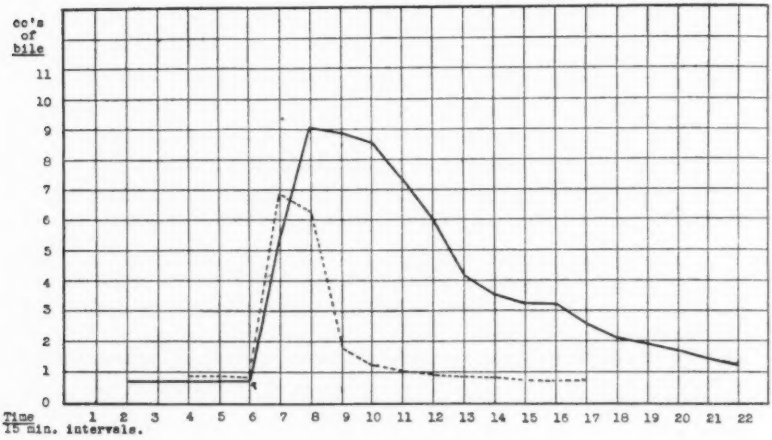

Fig. 2. Bile production following injection of bile salts.

↑ = Injection.

———— Bile production following intravenous injection of 2 grams of sodium dehydrocholate. Average for seven dogs.

---------- Bile production following intravenous injection of 2 grams of sodium glycocholate. Average for two dogs.

Bile was collected at fifteen minute intervals by means of a cannula in the common duct, the cystic duct being ligated. Control periods were run for at least one hour. Sodium dehydrocholate was then injected.

As shown in table 1 there was an increase in bile flow from 4.8 to 19 times the basal, the average being about 10 for 2½ hours following the injection in

TABLE 2

The effects of bile acids on total solids

DOG NUMBER	GRAMS OF SOLIDS PER 100 CC. OF BILE BEFORE Na DEHYDROCHOLATE	GRAMS OF SOLIDS PER 100 CC. OF BILE AFTER Na DEHYDROCHOLATE	PERCENTAGE CHANGE IN TOTAL SOLIDS
344	8.15	4.51	
	8.05	3.91	
	7.83	4.06	
	Av. 8.01	Av. 4.12	−48.57
345	7.91	3.77	
	8.88	3.74	
		3.80	
	Av. 8.39	Av. 3.77	−55.07
346	7.17	3.35	
	7.17	3.12	
	7.40	2.94	
		2.63	
		2.62	
	Av. 7.25	Av. 2.93	−59.60
347	7.94	4.09	
	6.56	4.21	
		3.67	
		3.52	
		2.97	
	Av. 7.25	Av. 3.69	−49.13
348	11.98	3.90	
	9.84	3.47	
		2.88	
	Av. 10.91	Av. 3.42	−68.66
350	7.70	4.02	
	7.64	4.10	
		2.60	
	Av. 7.67	Av. 3.57	−53.46
351	11.04	4.08	
		3.96	
		3.45	
		Av. 3.83	−65.31

seven dogs. The bile began to flow faster within one minute after injection. The length of time required for the bile flow to return to normal was from 2 to 5 hours, the average being 3½ hours.

Figure 2 is a graph of the bile flow showing the average for seven dogs. In all experiments with exception of one the bile changed from a dark greenish brown to a clear amber color. The one changed to a clear red color—resembling hemolyzed blood.

Total solid determinations were run on these samples (see table 2). There was a marked decrease in solids, more marked in the later samples.

Bile was collected at 15 minute intervals from two dogs. After obtaining the basal flow for over an hour we injected into one, a female weighing 20 kilograms, 10 cc. of a 20 per cent solution of sodium dehydrocholate, and into the other, a male weighing 26 kilograms, 10 cc. of a 20 per cent solution of sodium glycocholate (Gorlitz). In both cases there was an increase in the amount of bile produced per 15 minute interval. When bile flow had returned to nearly basal we injected sodium glycocholate in the dog

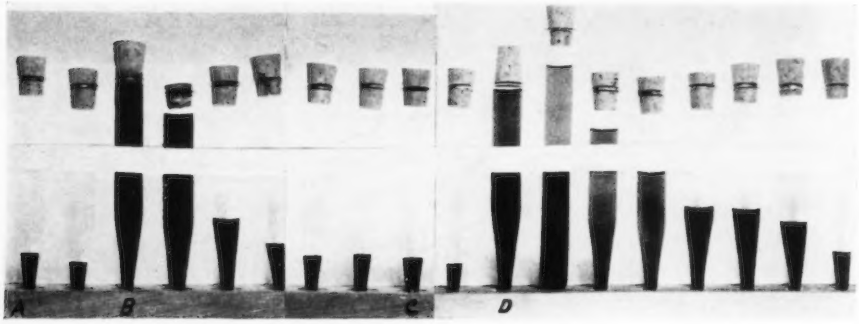

Fig. 3. Amounts of bile collected per 15 minute interval.

A, basal bile flow; *B*, bile flow following the intravenous injection of 2 grams of sodium glycocholate; *C*, basal bile flow; *D*, bile flow following the intravenous injection of 2 grams of sodium dehydrocholate.

which had previously received sodium dehydrocholate, and vice versa. There was an immediate increase in bile flow. In both dogs, as may be readily seen in the photographs of the tubes containing the bile collected per 15 minute interval, the sodium dehydrocholate caused a greater flow of bile and had a more prolonged action than the sodium glycocholate.

Bile pressure determinations were made on seven dogs by connecting a cannula in the common duct with a bromoform manometer. The cystic duct was ligated in each case. The bile was allowed to reach its maximum secretion pressure and then 10 cc. of 20 per cent sodium dehydrocholate injected intravenously. The average normal bile pressure was found to be 341 mm. of water. As a result of the injections the average pressure rose to 385 mm. of water or a 13 per cent increase.

Hemolysis experiments were run on sodium dehydrocholate according

to the method of Ponder, using human corpuscles. We found that it, in comparison with some very pure sodium glycocholate in similar concentrations, has very slight hemolytic effect.

Though all our data tend to show that dehydrocholic acid is much less toxic than glycocholic acid we are of the opinion that it does produce some toxic effects in dogs, in the doses which we have used. We have shown that it produces toxic effects even when such small quantities as 0.024 gram per kilogram are given. Neubauer concluded from his work on one dog that it was non-toxic in quantities up to 0.4 gram per kilogram when given intravenously. He does not give the rate of injection. The amounts of dehydrocholic acid used in these experiments were relatively much greater than that advised for the human.

SUMMARY

1. Dehydrocholic acid is not as toxic for frogs as cholic acid.

2. Dehydrocholic acid has toxic effects on dogs when given intravenously—but not as marked as those produced by glycocholic acid.

3. Dehydrocholic acid causes a fall in blood pressure, more prolonged but not as great as that produced by similar quantities of glycocholic acid.

4. Dehydrocholic acid causes a marked increase in bile flow—greater and more sustained than that produced by glycocholic acid. Bile secretion pressure is also increased.

5. Dehydrocholic acid has little effect on respiration.

6. Dehydrocholic acid causes an absolute increase but a relative or percentage decrease in total solids in the bile.

7. Dehydrocholic acid is hemolytic for red corpuscles only in high concentrations.

The writers are indebted to Dr. A. J. Carlson, under whose direction this work was conducted.

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DEMONSTRATION OF THE ACCELERATOR NERVE AND OF POSTGANGLIONIC PARASYMPATHETIC FIBERS IN THE VAGO-SYMPATHETIC TRUNK OF THE DOG

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During the latter part of the nineteenth century several investigators described an accelerator nerve in the vago-sympathetic trunk in mammals (Schmiedeberg, 1871; Boehm, 1875; Schiff, 1878; Bayliss, 1920; Arloing, 1896, and others). Later investigations on the cardio-accelerator action of the mammalian vagus have given conflicting results.

Tulgan (1923) found that when the vagus endings in the heart were paralyzed in the cat by the use of atropine, there remained in the vagus nerve fibers which cause an acceleration of the heart rate. Dale, Laidlaw and Symone (1910) also demonstrated an accelerator action in the vagus of the cat. They found that acceleration of the heart could still be produced by stimulating the vagus after the cervical sympathetics had been removed. These investigators conclude that the reaction is due either to a reversal of function of normally inhibitory fibers or to the presence of masked accelerator fibers belonging to the vagus. Stewart (1918) believes that the vago-sympathetic trunk in the dog usually carries some cardio-accelerator fibers. Reed and Layman (1930) found that stimulation of the peripheral end of the cut vagus in the dog with a weak tetanizing current sometimes caused an acceleration of the heart and a rise in blood pressure while a stronger current gave the typical vagus response.

Gaskell (1920) states that without exception, the cardio-motor fibers arise from the thoracico-lumbar divisions of the autonomic nervous system. After extensive investigations on the subject Hering (1924) concludes that the vagus or sympathetic trunk in the neck contains no accelerator fibers in the dog, cat, rabbit or monkey, although he admits that a weak stimuli to the intact vagus (especially on the right side) may accelerate the heart. Hering (1914) believes this to be due to a reflex action.

Two investigators have suggested the presence in the vagus of still another mechanism which probably represents postganglionic para-sympathetic neurones having their cell bodies in the nodose ganglia. According to Winkler (1918) the nodose ganglion is to be considered as the autonomic vertebral ganglion. It is here that the preganglionic course of the

autonomic fibers of the vagus and accessory nerves provisionally terminate. Koreiša (1931) found that after the vagus had been sectioned intracranially, and the dorsal nuclei allowed to degenerate, there remained vagus fibers beginning in the nodose ganglion which carry inhibitory impulses to the heart.

It is the purpose of the authors to present additional evidence concerning the presence of the accelerator nerve in the vagus of the dog. We have also obtained considerable evidence in support of the view that there are sometimes present in the vago-sympathetic trunk postganglionic fibers (especially on the left side) which, when stimulated, cause a fall in blood pressure and sometimes a slowing of the heart.

MATERIAL AND METHODS. As far as possible, only healthy young dogs were used for this investigation. In 25 animals the operation was successfully performed and adequate records obtained. Of this number 14 were used for a study of the left vagus and 11 were used for a study of the right vagus. First the animal was anesthetized and either the right or left vagus nerve cut proximal to the nodose ganglion as close as possible to the point where the vagus emerges from the jugular foramen. In three cases a small arterial clamp was placed on the nerve in this position instead of the nerve being severed. With two exceptions the animal was allowed to live for a period of 11 to 14 days. This period was calculated to allow ample time for the cut fibers to degenerate sufficiently so that they would not respond to electrical stimulation. As a check, two animals were allowed to live for only 8 days and it was found that the vagus did not respond to stimulation at the end of this shorter period.

Some difficulty was experienced in controlling hemorrhage due to blood vessels which run within the sheath of the vagus. It was found that this hemorrhage could be avoided by placing a small hemostat on the vagus at its point of emergence from the jugular foramen and cutting the nerve distal to the hemostat. The hemostat was then allowed to remain in position for a few minutes after the nerve was cut. Care was exercised in exposing the nerve only at the point at which it was to be cut and in avoiding injury as far as possible to the nodose ganglion.

Eleven to fourteen days after the operation the dog was placed under ether anesthesia and prepared for a study of the functional activity of the vagus which had previously been cut. A cannula was placed in the carotid artery on the unoperated side and connected to a mercury manometer arranged to record the heart rate and blood pressure on a smoked drum. The respiration was also recorded by means of a tracheal cannula connected to a respiratory tambour. A signal magnet was arranged to write on the smoked drum and connected in the primary circuit with an induction coil. The strength of the current was occasionally varied during the experiments but a tetanizing current of moderate strength was usually found

to be most effective in producing a response in the vagus. The vagi nerves were then exposed in the neck region and a series of records taken while either the normal or cut nerve was being stimulated. After the intact nerve had been stimulated the nerve was then cut in most cases and the peripheral and central ends stimulated.

A post-mortem examination was made to determine if the vagus had been completely severed proximal to the nodose and the nodose ganglion on the operated side was sectioned and stained for histological study.

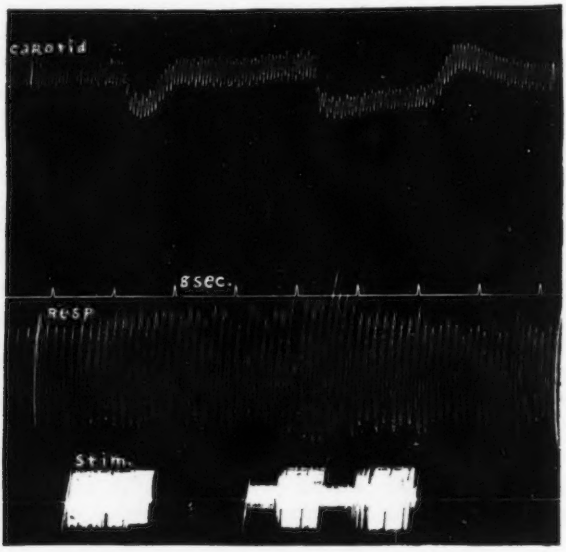

Fig. 1. Case 1. A record of the blood pressure and heart rate during stimulation of the left vago-sympathetic trunk. The left vagus had been cut proximal to the nodose ganglion 13 days previous.

Discussion. After the vagus has been sectioned proximal to the nodose ganglion and the cut fibers allowed to degenerate the changes in heart rate and blood pressure obtained by stimulating the vago-sympathetic trunk are illustrated in figures 1 to 3.

Figure 1 (case 1, left vagus) illustrates the fall in blood pressure which was usually obtained with the left vagus and occasionally with the right. With the first stimulus of 11 seconds' duration there was a latent period of 8 seconds after which the blood pressure fell from 146 mm. to 122 mm. and remained at that level for the remaining 3 seconds during which the stimulus was applied. With the fall in pressure the heart rate decreased from 127

to 120 beats per minute. With a second stimulus of 22 seconds' duration there was a latent period of 8 seconds; the blood pressure fell from 148 mm. to 122 mm., while the heart rate increased from 127 to 135 beats per minute.

Figure 2 (case 3, left vagus) is a record illustrating the response obtained when the vago-sympathetic trunk was cut in the neck and the peripheral end stimulated. With a stimulus of 20 seconds' duration the blood pressure fell from 120 mm. to 98 mm. The heart rate decreased from 150 to 127 beats per minute.

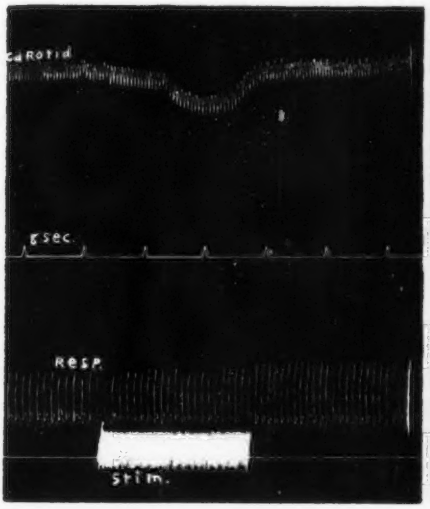

Fig. 2

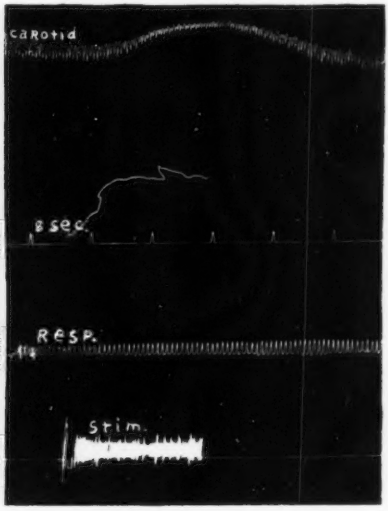

Fig. 3

Fig. 2. Case. 3. A record of the blood pressure and heart rate during stimulation of the peripheral end of the cut vagus on the left side. The vagus had been severed proximal to the nodose ganglion 14 days previous.

Fig. 3. Case 11. A record of the blood pressure and heart rate during stimulation of the left vago-sympathetic trunk. The vagus had been cut proximal to the nodose ganglion 12 days previous.

Figure 3 (case 11, left vagus) illustrates a second type of response frequently obtained on either the right or left side. In this case when the vago-sympathetic trunk was stimulated for a period of 18 seconds, the blood pressure rose from 126 mm. to 140 mm. and the heart rate increased from 138 to 168 beats per minute.

A study of the experiments described above shows that after the vagus is severed from its connection with the medulla, proximal to the nodose

ganglion, there may still remain in the vago-sympathetic trunk two active groups of motor fibers. One of these fiber groups, upon stimulation, causes a fall in blood pressure and frequently a slowing of the heart rate. A second group of fibers resembles the accelerator nerve which has been previously described by other investigators.

Among the 14 animals, in which the left vagus was cut above the nodose ganglion, stimulation of the left vagus caused a fall in blood pressure in 10 cases (cases 1 to 10). In 4 of these cases (cases 2, 4, 5, 7) there was sometimes a rise in blood pressure during stimulation while at other times there was a fall in pressure. In one case (case 11) a rise in blood pressure was the only response to stimulation. In 3 cases (cases 12, 13 and 14) there was no response to stimulation of the left vagus.

Among the 11 animals in which the right vagus was cut above the nodose ganglion, only two (cases 15 and 16) showed a slight fall in blood pressure when the right vagus was stimulated. In these two cases there was sometimes a rise in blood pressure during stimulation. In fact, a rise in blood pressure was more frequently elicited and more pronounced than the fall in pressure which sometimes occurred. In 3 cases (cases 17, 18 and 19) a rise in blood pressure was the only response to stimulation of the right vagus.

From the above summary it will be seen that the neurone which is responsible for lowering blood pressure is apparently present much more frequently on the left side than on the right. On the left side a lowering of blood pressure was obtained in 71 per cent of the cases studied. On the right side a lowering of the pressure only occurred in 18 per cent of the cases. While our series of animals is too small and the variability in the response to stimuli too great to enable us to state that this mechanism is normally present in a definite percentage of cases, these experiments do indicate a difference between the right and left side. The so-called accelerator nerve in the vagus which causes an acceleration of the heart and a rise in blood pressure was demonstrated in almost the same percentage of cases on the right and left sides.

There are two possible factors concerned with the fall in blood pressure which occurs with stimulation of the vagus nerve. The first of these is a slowing of the heart which may result in a decrease in the output of blood from the heart. The second factor which may play a rôle in lowering blood pressure is a dilatation of the blood vessels in the viscera. Although we have no positive proof of the relative importance of these two factors in our operated animals, an examination of the blood pressure tracings may be of interest. In those cases in which the left vagus was cut proximal to the nodose ganglion and the animal allowed to live for 12 to 14 days, the results were as follows:

Of the records which showed a definite lowering of blood pressure when

the left vagus was stimulated, there was a definite slowing of the pulse in six instances. Three of the tracings showed a lowering of pressure with no change in pulse rate, while in three instances the pulse rate was accelerated. In both of those cases in which stimulation of the right vagus caused a fall in blood pressure, there was a marked acceleration of the pulse rate. These observations suggest the probability that dilatation of the blood vessels in the viscera plays a more important rôle in lowering the blood pressure in these cases than does a slowing of the heart rate.

In those cases in which there was a rise in blood pressure following stimulation of the vagus on the operated side, the rise in pressure was accompanied by a marked increase in pulse rate in all but two cases. In cases 17 and 18 there was a rise in blood pressure with no apparent change in heart rate. The accelerator nerve was demonstrated in five cases on the left side and five on the right.

It will be noted that the parasympathetic neurone, which remains intact after the vagus has been severed from the medulla, responds to stimulation in a manner quite different from the normal vagus. While the normal vagus responds very quickly to stimulation and the blood pressure falls suddenly to a low level, the operated vagus responds more slowly, usually after a latent period of a few seconds. In the operated animals it was sometimes necessary to stimulate the nerve for several seconds and to repeat the stimulation a number of times before a response could be elicited. The lowering of the blood pressure usually proceeded gradually, was rather prolonged and then was slow in returning to normal. By means of the potential record Bishop and Heinbecker (1930) have distinguished four types of fibers in the vagus nerve. These fiber groups differ in conduction rates and in such properties as threshold, chronaxie, refractory period, and duration of axon potential response.

A histological and cytological study was made of the nodose ganglia on the side on which the vagus had been previously sectioned, proximal to the ganglia. Only a slight amount of chromotolysis was present among the ganglion cells and that was not confined to any one type of cell. This study gave no definite evidence which could be applied to our problem.

Although our investigation indicates that an accelerator nerve can frequently be demonstrated in the vago-sympathetic trunk of the dog, the origin of this nerve is still unknown. After section of the vagus proximal to the nodose ganglia there remains an intact neurone (especially on the left side) which is capable of lowering blood pressure and slowing the heart. We are probably dealing here with a postganglionic parasympathetic neurone. The cell body of this neurone is probably located in the nodose ganglion. According to Kure, Nitta, Tuzi, Siraisi and Suyenaga (1918) there are postganglionic parasympathetic neurones with their cell bodies located in the dorsal root ganglia of the spinal cord. These authors review

the work of Imagawa, Sunago, Kozima and Sakurazawa which indicates that similar parasympathetic fibers run in the chorda tympani, and that there are postganglionic parasympathetic fibers having their cells of origin in the ciliary and sphenopalatine ganglia. In addition to the five cell types which are present in spinal ganglia, Clark (1926) found 90 per cent of the cells of the nodose ganglia to be of another type (type F) which is not present in the spinal ganglia. A majority of the cells in the jugular, glossopharyngeal and geniculate ganglia were also found to belong to class F. Molhant's (1912) localization of cell groups in the nodose ganglia, according to the regions which they innervate, does not correspond to Clark's grouping which was based on cell type.

Much additional work must be done before we can understand the vagus and cervical sympathetics and the rôle which these mechanisms play in the regulation of heart rate, peripheral circulation and other vegetative functions.

SUMMARY

When the vagus nerve of the dog was cut proximal to the nodose ganglion and the cut fibers allowed to degenerate, two distinct groups of intact nerve fibers could still be demonstrated in the vago-sympathetic trunk.

The first of these fiber groups resembles the accelerator nerve of other investigators. Stimulation of this nerve causes an acceleration of the heart and a rise in blood pressure. It was demonstrated in 40 per cent of the animals studied.

The second group of fibers present in the vago-sympathetic trunk after vagotomy can probably be interpreted as being postganglionic parasympathetic fibers with their cells of origin in the nodose ganglia. Stimulation of these fibers results in a lowering of the blood pressure and sometimes a slowing of the heart. These fibers were demonstrated on the left side in 71 per cent of the cases studied and were present on the right side of 18 per cent of the cases used for a study of the right vagus.

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STUDIES ON SUPRARENAL INSUFFICIENCY

X. Depressor Responses to Small Doses of Adrenalin in the Rat, Induced by Loss of the Suprarenal Medulla

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The suggestion has been made in the past that adrenin may have a continuous effect maintaining the nutrition or tone of some part of the vascular neuro-muscular mechanism. For such a view we have found no evidence in our previous work; nor is it in accord with Cannon's recent report of "evidence that medulliadrenal secretion is not continuous" (1931). This suggestion, however, led us to examine the vascular responses to adrenalin of normal rats and of suprarenalectomized rats which were kept in good health by transplants of cortical tissue but had been lacking the suprarenal medulla for some time. In a survey of the effects of injected adrenalin on the vascular mechanism of a great variety of animals, Hartman, Kilborn and Lang (1918) came to the conclusion that rodents are exceptional in their reaction to adrenalin, having no vasodilator mechanisms sensitive to this substance. They could not obtain a fall of blood pressure in the white rat with adrenalin, responses to all effective doses being pressor. We confirmed this finding in urethanized normal rats, but in the transplanted rats without suprarenal medulla the responses to small doses were consistently depressor. This phenomenon was so unusual that further work was done and the results are reported below.

Methods. The general technique of studying suprarenal insufficiency in rats has been described in previous papers in this series. Details regarding methods of recording blood pressure, intravenous injections, and microscopic observation of blood vessels in rats may be found in the paper by Wyman and tum Suden (1932). Carotid blood pressures were measured by the mercury manometer method and intravenous injections were given in the femoral vein. Adult male rats, between 230 and 300 grams' body weight, were used throughout.

Normal rats. In a series of 17 normal male rats, anesthetized with urethane, with initial blood pressures ranging from 90 to 134 mm. Hg (average 105.4 mm. Hg), the minimal effective doses of adrenalin produced purely

pressor effects (fig. 1). In five cases such a dose was 0.05 cc. of 1:2,000,000, in nine cases 0.05 cc. of 1:1,000,000, in two cases 0.1 cc. of 1:1,000,000, and in one case 0.05 cc. of 1:4,000,000 produced a detectable pressor effect. In 10 rats anesthetized with ether with initial blood pressures from 102 to 118 mm. Hg (average 111.8 mm. Hg), similar minimal doses produced only pressor effects in four cases (fig. 12, A). In the other six cases the smallest effective doses (0.05 cc. of 1:2,000,000 to 0.05 cc. of 1:1,000,000) produced depressor responses, larger doses (0.05 cc. to 0.1 cc. of 1:1,000,000) produced pressor responses, and intermediate doses produced pressor-depressor effects (fig. 11, A). When depressor responses were obtained the fall of blood pressure produced by a given dose was reduced by decreasing the depth of the ether anesthesia just before the injection, and it was increased by administering more ether before the injection. Wyman and Lutz (1925) found that the pressor response to adrenalin in cats may be modified in various ways by varying the dosage of inhaled ether.

In these and in all subsequent experiments adequate control injections were given, of normal saline solution or of saline solution containing chloretone in amounts as great as or greater than that contained in the adrenalin chloride solution used (Parke, Davis & Co.).

Cortical transplants. In five suprarenalectomized rats having autoplastic transplants of cortical tissue but no demonstrable chromaffin tissue and two suprarenalectomized rats having gross accessory cortical tissue, all exhibiting normal blood pressures under urethane anesthesia (104 to 122 mm. Hg, average 111.7 mm. Hg), definite depressor responses were regularly obtained with small doses of adrenalin, i.e., from 0.02 cc. to 0.1 cc. of 1:2,000,000 (figs. 2 and 3). Larger doses, from 0.1 cc. of 1:1,000,000 to 0.05 cc. of 1:500,000 or more, produced brief rises of blood pressure immediately followed by prolonged falls. Much larger doses, from 0.1 cc. of 1:500,000 to 0.05 cc. or 0.1 cc. of 1:100,000, were required to produce purely pressor effects. Exactly the same course of events was observed in three etherized suprarenalectomized rats having gross accessory cortical tissue, four months after operation (fig. 4). The depressor responses seen in these animals were much greater than any observed in normal rats under ether anesthesia, and large doses of adrenalin (0.1 cc. of 1:100,000), such as invariably produce marked pressor effects in etherized normal rats, caused pressor-depressor responses. Under urethane anesthesia, at least, there is apparently a complete reversal of the vascular responses to small doses of adrenalin in suprarenalectomized rats having cortical but no medullary suprarenal tissue. Such animals are perfectly healthy, have abundant body fat, and apparently differ from normal rats only in respect to the medullary defect.

Suprarenalectomized rats. Experiments were done with urethane anesthesia on nine suprarenalectomized rats which showed absence of all gross

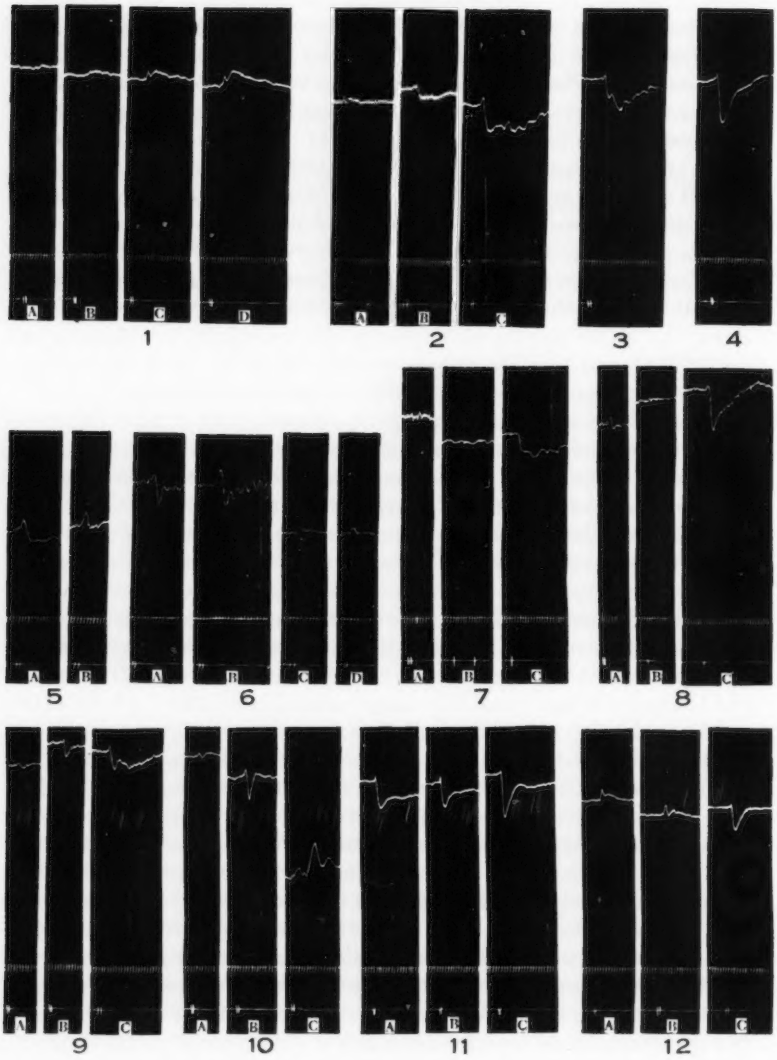

Fig. 1. Experiment 76. Normal male rate, 291 grams. Urethane anesthesia. In this, and in subsequent tracings, the time record shows five second intervals and is at zero blood pressure level. The lower record signals intravenous injections (femoral vein) of adrenalin solution or control injections of normal saline solution,

with or without chloretone. A, 0.1 cc. chloretone in normal saline, 1:500,000; B, 0.1 cc. adrenalin, 1:2,000,000; C, 0.1 cc. adrenalin, 1:1,000,000; D, 0.1 cc. adrenalin, 1:500,000.

Fig. 2. Experiment 62. Male rat, 276 grams, having transplanted cortical tissue but no demonstrable chromaffin tissue. Urethane anesthesia. A, 0.1 cc. and 0.05 cc. saline solution; B, 0.1 cc. adrenalin, 1:2,000,000; C, 0.05 cc. adrenalin, 1:500,000.

Fig. 3. Experiment 65. Male rat, 274 grams, transplant. Urethane anesthesia; 0.1 cc. adrenalin, 1:1,000,000.

Fig. 4. Experiment 98. Male rat, 298 grams, 124 days after suprarenalectomy, having gross accessory cortical tissue. Ether anesthesia; 0.05 cc. adrenalin, 1:1,000,000.

Fig. 5. Experiment 43. Male rat, 249 grams, 16 days after suprarenalectomy. Urethane anesthesia. A, 0.1 cc. adrenalin, 1:2,000,000; B, 0.1 cc. adrenalin, 1:1,000,000. Low blood pressure (52 mm. Hg), pressor action predominating.

Fig. 6. Experiment 38. Male rat, 301 grams, 10 days after suprarenalectomy. Urethane anesthesia. A, 0.05 cc. adrenalin, 1:1,000,000; B, 0.1 cc. adrenalin, 1:1,000,000 (blood pressure medium, 80 mm Hg; pressor-depressor action); C, 0.05 cc. adrenalin, 1:1,000,000; D, 0.1 cc. adrenalin, 1:1,000,000 (31 minutes after dose B; blood pressure low, 51 mm. Hg; pressor action only).

Fig. 7. Spinal cord transected under ether anesthesia, ether discontinued, blood pressure from femoral artery. A, Experiment 114. Normal male rat; 0.1 cc. adrenalin, 1:2,000,000, 44 minutes since discontinuing ether. B, Experiment 116. Male rat, 308 grams, transplant. 0.05 cc. and 0.1 cc. chloretone solution, 1:500,000, 38 minutes since ether. C, Experiment 116; 0.1 cc. adrenalin, 1:2,000,000, 46 minutes since ether.

Fig. 8. Decerebrated under ether anesthesia, ether discontinued, blood pressure from carotid artery. A, Experiment 119. Normal male rat, 304 grams; 0.1 cc. adrenalin, 1:1,000,000, 40 minutes since discontinuing ether. B, Experiment 118. Male rat, 260 grams, transplant. 0.1 cc. chloretone solution, 1:500,000, 35 minutes since ether. C, Experiment 118; 0.1 cc. adrenalin, 1:1,000,000, 54 minutes since ether.

Fig. 9. Experiment 87. Male rat, 285 grams. Urethane anesthesia. Suprarenals ligated and removed before the experiment. A, 0.1 cc. chloretone solution, 1:100,000, 90 minutes since suprarenalectomy. B, 0.1 cc. adrenalin, 1:2,000,000, 44 minutes since suprarenalectomy. C, 0.1 cc. adrenalin, 1:1,000,000, 48 minutes since suprarenalectomy.

Fig. 10. Experiment 97. Normal male rat, 240 grams. Urethane anesthesia. A, 0.1 cc. adrenalin, 1:1,000,000. Suprarenalectomy 20 minutes after dose A. B, 0.1 cc adrenalin, 1:1,000,000, 16 minutes after suprarenalectomy. C, 0.1 cc. adrenalin, 1:1,000,000, 126 minutes after suprarenalectomy (blood pressure low, 54 mm. Hg; pressor action only).

Fig. 11. Experiment 108. Normal male rat, 278 grams. Ether anesthesia. A, 0.1 cc. adrenalin, 1 1,000,000. Control blank operation, 8 minutes later. B, 0.1 cc. adrenalin, 1:1,000,000, 9 minutes after blank operation. Suprarenals ligated 5 minutes after dose B. C, 0.1 cc. adrenalin, 1:1,000,000, 10 minutes after ligation of suprarenals. Increased depressor response.

Fig. 12. Experiment 110. Normal male rat, 250 grams. Ether anesthesia. A, 0.1 cc. adrenalin, 1:1,000,000. Control blank operation 5 minutes later. B, 0 1 cc. adrenalin, 1:1,000,000, 21 minutes after blank operation. Suprarenals ligated 45 minutes after dose B. C, 0.1 cc. adrenalin, 1:1,000,000, 9 minutes after ligation of suprarenals. Reversal of response.

suprarenal tissue at autopsy, from 10 to 17 days after operation in six cases and 99 or 101 days after operation in three cases. Such animals have a low blood pressure concurrent with the cortical defect (Wyman and tum Suden, 1932). In four cases with blood pressures between 38 and 58 mm. Hg only pressor responses to adrenalin were observed (fig. 5). The threshold dosage was the same as that for normal rats. In five cases with blood pressures between 60 and 80 mm. Hg depressor or pressor-depressor responses were obtained with the smallest effective doses (0.1 cc. of 1:2,000,000 or 1:1,000,000), and purely pressor effects with larger doses, as in the case of the transplanted rats, except that somewhat smaller doses sometimes produced purely pressor effects (fig. 6, A, B). This is consistent with the hypothesis proposed by Cannon and Lyman (1913), namely that the action of adrenalin on vascular smooth muscle varies according to the state of the muscle, being relaxation when the muscle is tonically shortened and contraction when relaxed. They observed that the depressor effect of small doses of adrenalin in the cat was reversed to pressor action when the blood pressure was lowered by various means. The gradation of depressor, through pressor-depressor, to purely pressor effects produced by the same dose of adrenalin in suprarenalectomized animals with different decreasing blood pressure levels is striking. That these differences are dependent upon the blood pressure level is further substantiated by reversal of the depressor to a pressor-depressor or pressor action in the same animal when the blood pressure falls during an experiment. Such a reversal was seen in eleven cases (fig. 6, C, D; fig. 10, C).

Acute experiments. In order to determine whether the reversal of the vascular response to a small dose of adrenalin is dependent upon a chronic condition developed in suprarenalectomized rats some time following operation or whether it appears immediately following the removal of the influence of the suprarenal medulla, a variety of experiments was done, using both urethane and ether anesthesia.

A control blank operation in the suprarenal sites accompanied by more trauma than that involved in double suprarenalectomy, or single suprarenalectomy together with a blank operation on the opposite side did not alter the blood pressure or the vascular responses to intravenous injections of various doses of adrenalin given from 2 to 72 minutes later (fig. 11, A, B; fig. 12, A, B). Such operations were done in ten cases, either before the blood pressure experiment was begun or during the recording of blood pressure after a number of test doses of adrenalin had been given.

Six urethanized rats were suprarenalectomized from 20 to 194 minutes before recording blood pressure. These animals had normal blood pressures and responded to intravenous adrenalin, injected from 35 to 209 minutes after suprarenalectomy, in the same way as the transplanted rats described above (fig. 9).

In a series of twelve rats, three urethanized and nine etherized, the suprarenals were carefully ligated during the experiment, after a number of test doses of adrenalin had been given. In six of these cases ligation of the suprarenals had been preceded by a control blank operation (from 12 to 79 minutes previously), so that the necessary incisions had already been made and the ligation involved a minimum of operative trauma. In these cases also, the normal control injections of adrenalin, those following blank operation, and those following suprarenal ligation were all carried out in succession in the same animal and were thus quite comparable. In all cases simple ligation of the suprarenal glands, with no accompanying alteration of blood pressure, was followed by reversal of the vascular responses to small doses of adrenalin. Doses which had produced purely pressor responses before the ligation and whose action had not been altered by the control operation now produced definite depressor responses (figs. 10 and 12). The reactions to increasing doses of adrenalin were the same as those seen in the transplanted rats or in rats suprarenalectomized shortly before the blood pressure experiment. In four cases, under ether anesthesia, the smallest doses had produced depressor responses before suprarenal ligation. In these cases, following the ligation, similar doses produced much greater depressor responses, the fall of blood pressure sometimes being twice as great as previously (fig. 11). Depressor responses to small doses were obtained from 2 to 108 minutes after ligation of the suprarenals. Evidently the reversal occurs immediately following the removal of the influence of the suprarenal medulla, and is constant for a comparatively long time. Judging from the data obtained from transplanted animals, tested four months after suprarenalectomy, it is a permanent reversal.

Experiments without anesthesia. In 1926 Macdonald and Schlapp reported that in the unanesthetized decerebrate cat no depressor responses to small doses of adrenalin could be evoked, and in 1928 Dragstedt, Wightman and Huffman found that in the normal unanesthetized dog the minimal effective dose of adrenalin on sustained administration produces pressor effects. The opinion became prevalent, therefore, that the depressor response to a small dose of adrenalin is an effect conditioned by anesthesia. Gruber (1928) found, nevertheless, that small doses of adrenalin cause a fall of blood pressure in decerebrated cats from 30 minutes to 3½ hours after withdrawal of the anesthetic. It became necessary, therefore, to see if the depressor response in rats lacking the suprarenal medulla is obtained only in anesthetized animals.

Two types of experiment were done. In one the spinal cord was sectioned in the mid-lumbar region under ether anesthesia, the rat was fastened as comfortably as possible to an operating board, the ether was discontinued, and blood pressure was recorded from the femoral artery. In the other the

mid-brain was sectioned under ether anesthesia through a small hole in one side of the skull after ligating the carotid arteries, the ether was discontinued, and blood pressure was recorded from the carotid artery as usual. Both types were done in the case of normal and of suprarenalectomized rats having autoplastic cortical transplants but no medullary tissue three months after transplantation. In all cases the blood pressures were within the normal range after preparation of the animal and during the experiment (104 to 136 mm. Hg).

The reactions to various amounts of adrenalin injected intravenously were the same in all cases as those observed in urethanized rats and described above. In normal rats the minimal effective doses, given from 25 to 144 minutes after discontinuing ether, were purely pressor in action and were of the same general magnitude as those for anesthetized rats. In transplanted rats small doses, given from 32 to 64 minutes after discontinuing ether, caused depressor responses, and the size of the effective doses and of the depressor responses were again similar to those characteristic of anesthetized rats (figs. 7 and 8). Evidently the phenomenon in question is not conditioned by anesthesia.

Heart rate. Records were taken on a fast drum during various types of experiment and in the case of the different types of animal. Counts showed that no significant changes in heart rate were caused by the control injections of saline or of chloretone solutions, or by the small doses of adrenalin.

Direct observation of blood vessels. Direct microscopic observation of the blood vessels of the ear by transmitted light and of the vessels of the intestines by reflected light through a celluloid abdominal window (Mendenhall, 1931) was carried out on normal and on transplanted rats during the intravenous injection of various amounts of adrenalin. In the case of the ear vessels of both types of rat only vasoconstriction (veins and arteries) was observed, and the minimal effective dose of adrenalin was 0.1 cc. of 1:500,000. In the case of the vessels on the surface of the intestine or in the mesentery of normal rats the minimal dose which caused changes of sufficient magnitude to be definitely interpreted was 0.05 cc. of 1:500,000, and produced constriction only. Larger doses, from 0.1 cc. of 1:500,000 to 0.05 cc. of 1:10,000, produced constriction only or predominating constriction followed by slight dilatation. In transplanted rats doses of from 0.1 cc. of 1:1,000,000 to 0.1 cc. of 1:100,000 caused brief constriction followed by a marked, prolonged dilatation. Larger doses caused marked constriction followed by a comparatively brief dilatation. Evidently the depressor phenomenon induced by loss of the suprarenal medulla is of vascular origin. The observations on the normal rats are in accord with those of Hoskins, Gunning and Berry (1916) on the dog with respect to cutaneous vessels, and are similar to those of Hartman and McPhedran

(1917) on dogs and cats, namely, that small doses cause constriction of intestinal vessels while larger doses cause constriction followed by dilatation.

DISCUSSION. From the results reported above it may be concluded that the generalization stating that rodents do not possess vasodilator mechanisms sensitive to adrenalin is not strictly accurate. Although normal rats under urethane anesthesia or without anesthesia do not react to minimal effective doses by vasodepression, they occasionally show depressor responses to adrenalin under ether anesthesia. The depressor responses to small doses of adrenalin induced by loss of the suprarenal medulla indicate, moreover, that vasodilator mechanisms responding to adrenalin are present, in the rat at least, although their presence in the intact animal is usually masked by pressor reactions. The microscopic observations of blood vessels indicate that these depressor responses are caused by peripheral vascular dilatation, the splanchnic vessels but not the skin vessels being involved. No observations were made on the vessels of skeletal muscle. Experiments without anesthesia show that the responses are not dependent upon the presence of an anesthetic. From this work and from the work of others on decerebrated animals it may be concluded that it is not safe to make the generalization that all depressor responses to adrenalin are conditioned by anesthesia.

An attempt at explanation of the reversal of the vascular response in the rat to a small dose of adrenalin occasioned by removing the suprarenal medulla is difficult, because there is no general agreement as to the cause of depressor responses to adrenalin in other animals. As stated above, the results seen in suprarenalectomized and in other types of rats with low blood pressures are consistent with the hypothesis of Cannon and Lyman (1913) that the action of small amounts of adrenalin varies according to the state of the vascular smooth muscle. Hartman and his collaborators concluded that adrenalin has a differential action on various parts of the vascular tree, and that depressor responses after small doses may be explained by the predominance of vasodilatation in certain areas over vasoconstriction in others (see Hartman, 1918). There is much evidence from various sources to support the idea of the existence of sympathetic vasodilator depressor mechanisms.

The reversal from pressor to depressor action appears a few minutes after removal of the influence of the suprarenal medulla, and is apparently permanent. It might be postulated that in the intact animal the injection of a minute dose of adrenalin causes the immediate secretion of enough additional adrenin from the rat's own glands to make the resulting response pressor in nature. The brief latent period of the response, however, militates against an explanation involving the induction of secretory processes. Much work remains to be done before this phenomenon can be explained, and it is quite possible that some undetected factor other than removal of

the suprarenal medulla is present. As a working hypothesis, one involving differential change in irritability of pressor and depressor mechanisms seems more attractive.

There is no doubt that a great many factors, many of them chemical in nature, can modify the vascular reactions to adrenalin (Collip, 1922; Lutz and Wyman, 1925; Wyman and Lutz, 1925). Many of these probably operate by changing the irritability of some part of the peripheral neuro-muscular mechanism in a selective manner. Hoskins and Rowley (1915) found that intravenous infusion of adrenalin decreases the irritability of both the pressor and depressor mechanisms of the dog. They also found that the depression of irritability develops almost immediately after beginning the infusion, and that it disappears as quickly upon discontinuing the infusion. Although Cannon (1931) has recently presented evidence that in the normal resting animal adrenin is not secreted continuously, he points out that operative disturbance causes a considerably exaggerated medulliadrenal secretion; and that "on abolishing, by adrenalectomy or cutting the secretory nerves, the extra secretion induced under experimental conditions, characteristic atonic effects might result because the spontaneously secreted adrenin has been exerting a tonic influence." It is conceivable, therefore, that in the experiments reported above such a secretion of adrenin in normal rats, induced by the experimental procedure, is constantly depressing the irritability of the vasomotor mechanisms. If we assume that the vasodepressor mechanisms may be more profoundly affected than the pressor mechanisms, then removal of the suprarenals should allow their action to be more easily elicited, and depressor responses to small doses of adrenalin would be more likely to occur. Hence the reversal following ligation of the glands. In transplanted rats, with no medullary tissue, no such secretion of adrenin can occur, hence depressor irritability is not differentially decreased and depressor responses may be regularly obtained. As indicated above, such a conception is offered merely as a tentative hypothesis.

SUMMARY

1. In urethanized and in unanesthetized normal rats minimal effective intravenous doses of adrenalin produced only pressor responses. In etherized normal rats similar minimal doses occasionally produced depressor effects.

2. In suprarenalectomized rats having transplants of cortical tissue but no demonstratble chromaffin tissue (either urethanized, etherized or unanesthetized), small doses of adrenalin produced depressor responses, larger doses produced pressor-depressor responses and much larger doses were required to produce purely pressor effects.

3. In suprarenalectomized rats similar results were obtained unless the blood pressure was low, when pressor responses alone were seen.

4. Removal or simple ligation of both suprarenal glands was immediately followed by reversal from pressor to depressor of the vascular responses to small doses of adrenalin. The reactions to increasing doses were the same as those in transplanted rats.

5. No significant changes in heart rate were observed. Microscopic observation of blood vessels during the injection of adrenalin in normal and in transplanted rats showed only constriction of the ear vessels, predominating constriction of the intestinal vessels in normals, and brief constriction followed by marked dilatation of intestinal vessels in transplants.

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THE ELASTICITY OF THE DURAL SAC AND ITS CONTENTS

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A report, recently published by the writers (Weed, Flexner and Clark, 1932), presented evidence of the existence of an important relationship between the dislocation of cerebrospinal fluid and its pressure. This relationship was developed from information yielded by experiments dealing with the abrupt tilting of dogs from the horizontal to the vertical head-down and tail-down positions. In these former observations, the pressure of the cerebrospinal fluid in the occipital region was determined by successive use of the so-called "bubble manometer" and of open-end manometers of various bores. With the bubble manometer pressure-changes in the cerebrospinal fluid were recorded without external dislocation of fluid, but with the open-end series an obvious dislocation of fluid into or from the manometer occurred on such tiltings. Determination of the volume of fluid displaced into or from the external system showed that as a progressively larger dislocation of fluid occurred in the manometers of larger and larger bore, the pressure-changes in these manometers became smaller and smaller.

From the data assembled in these tilting experiments, it was found that the difference in volume of displaced fluid had a definite relationship to the difference in pressure-change. This relationship was expressed in the fraction $\frac{dV}{dP}$, where dV represented the difference in volume-change between the tilting with no external displacement (bubble manometer) and the actual cubic centimeter change in any of the open-end manometers, while dP represented the difference in centimeters of the pressure-change on tilting between that recorded by the bubble manometer and those of any of the open-end manometers. Calculation of this fraction $\frac{dV}{dP}$ gave a fairly constant value in experiments carried out on dogs of a uniform size (weight, 6–7 kgm.). The fraction, which in dogs of that size was found to have an average value of 0.17, was also derived by using the differences in the pressure-changes from any two of the open-end manometers for comparison with the corresponding volume-differences. Employment of

this factor $\frac{dV}{dP}$ with its average value enabled the writers accurately to determine in young dogs of the same size the decrease in the intradural contents effected by the intravenous injection of strongly hypertonic saline solutions (Weed and McKibben, 1919a, b).

It was pointed out in the previous report that this fraction $\frac{dV}{dP}$ was related to the general physical formula for an elastic system. The expression for the coefficient of elasticity of a solid, fluid or gas, is the quotient obtained by dividing the stress by the strain. In this formula for the coefficient of the volume-elasticity E, the stress is the change in pressure dP, and the strain is the change in volume dV divided by the original volume V; i.e., E = coefficient of elasticity $= dP \Big/ \frac{dV}{V} = \frac{dP}{dV} V$.

It was found that this formula could be applied to the dural sac and its contents as the volume-elasticity of the membranes and vascular bed apparently led to a dislocation of fluid dV which is related to a pressure-change dP on tilting. As the ratio $\frac{dV}{dP}$ was ascertained to be fairly constant in any one animal, where the total volume V was the same, the formula given above may be applied to calculation of the coefficient of elasticity when $\frac{dV}{dP}$ and V have been determined. This led us to compute the elasticity of the dural sac and its contents in the dog. The first step in each experiment consisted in the derivation of the value of the fraction $\frac{dV}{dP}$ for the individual animal by measurement of the pressure-changes in the cerebrospinal fluid, with manometers of different bore, on tilting from the horizontal to the vertical head-down and tail-down positions. This procedure was followed by measurement of the volume of the animal's intradural contents, both cranial and spinal. These determinations $\left(\frac{dV}{dP} \text{ and total volume } V\right)$ enabled us to derive the coefficient of elasticity in dynes per square centimeter by substitution in the formula $E = V \Big/ \frac{dV}{dP}$.[1]

METHODS OF INVESTIGATION. While in the previous series of experiments dogs of a uniform size (6–7 kgm.) were employed, the present series of observations is based upon dogs of various sizes and various ages. The dogs were graded, as far as possible, as immature, young adult, adult and old

[1] Pressure was measured in centimeters of normal saline solution (or cerebrospinal fluid); but in order to give the coefficient of elasticity in C. G. S. units, pressure which is defined as force (mass × acceleration) per unit area, must be expressed as dgh (density × acceleration of gravity × height in centimeters).

animals. They were all anesthetized with ether, given by Woulffe bottle with intratracheal tube, and were then firmly fixed to a tilting table. The pressure of the cerebrospinal fluid was measured by attachment of open-end manometers of various bores (1 mm., 4 mm., 6 mm., 8 mm., and 10 mm.) to a needle inserted through the occipito-atlantoid ligament into the subarachnoid space. Initially the 1 mm. manometer was connected to the puncture-needle and the animal tilted from the horizontal to the two vertical positions. The readings of this manometer were followed by the attachment of the other manometers, with similar tiltings carried out with each manometer. The pressure-changes in the two series of vertical tilts (head-down and tail-down) were thus determined and the volume of fluid dislocated into or from each manometer calculated from the known capacities of the manometers.

The bubble manometer, as used in the former experiments, was not employed in these observations, because of additional technical difficulties during the tiltings. The pressure-changes in the cerebrospinal fluid, obtained with the open-end 1 mm. manometer, were considered as the baseline for reference to the pressure-changes recorded by the manometers of larger bore.

At the conclusion of each experiment, the animal was killed and the total intradural volume determined. The simplest method of accurate measurement of this volume was found to consist first in the removal of the head from the vertebral column. The intradural contents of the cranium were washed out with a fine stream of water, after first destroying the integrity of the brain by a stiff wire. For the spinal determinations, the best procedure was found to consist initially of laminectomy in the lower lumbar and mid-thoracic regions, taking care not to injure the spinal dura. In the first few animals a complete laminectomy was done but it was later found that exposure in these two segments was sufficient. The speedy maceration of the spinal cord and the spinal leptomeninges was then achieved by pushing a blunt fusiform enlargement of a fairly stiff wire through the entire length of the spinal dural canal, after first opening the dural sac in the lower lumbar segment. The macerated tissues were next completely washed out by a jet of water from a fine rubber tube.

After complete removal of the substance of the nervous system, the cranial intradural cavity was filled with mercury and the contained mercury subsequently measured. This method was found to be accurate, though signs of undue pressure, due to the weight of the mercury, were occasionally apparent. The method was checked for accuracy in a few animals by filling the cavity with lead shot with later measurement of the volume of these shot.

The early determinations of the spinal intradural volume were made by this method of filling the dural sac with shot. The technical difficulties

of this method were so great that mercury was next tried. The mercury was found to be too heavy for this purpose, and finally the simple expedient of inserting a small cannula into the lower end of the lumbar dural sac was employed. Through this cannula into the spinal dural tube a colored mineral oil was allowed to run from a burette, the animal being held with the head-end elevated until the filling was complete. This method checked accurately against the laborious employment of lead shot in such wet preparations.

EXPERIMENTAL FINDINGS. It was early discovered that a constant degree of light surgical anesthesia was necessary for the successful completion of the experiments with repeated tilting. In the customary experiment the necessary adjustment of the ether was early achieved and accurate measurements of the pressure-changes of the cerebrospinal fluid were then obtained. Because of frequent disturbances in respiration (chiefly hyperpnea) when the animals were in the vertical positions, it was found expedient to pinch off the ether tube during these periods. With care taken to secure a uniform degree of light surgical anesthesia and elimination of difficulties due to increasing etherization in the vertical positions, experiments yielding comparable results were readily carried to completion.

With the open-end manometer of 1 mm. bore attached at the beginning of the experiments, tiltings from the horizontal to the two vertical positions were made at least twice for each position. When experimental conditions were well established, the pressure-changes in the cerebrospinal fluid on such tiltings were found to be quite similar, usually varying but 2 or 3 mm. In cases of marked variation in the pressure-changes, several additional tiltings were performed so that an average value for the pressure-change could be obtained. In each animal, after attachment of the next manometer in series, at least two tiltings to each of the two vertical positions were made before proceeding to the next larger manometer.

With the pressure-changes and the volume-changes determined for each of the manometers, it became possible to calculate the fraction $\frac{dV}{dP}$ for the individual animal. This derivation was made by taking as dP the difference between the pressure-change obtained by the use of the 1 mm. manometer on tilting, and the pressure-changes recorded by the other manometers. The volume-change dV represented the difference between the volume dislocated in the 1 mm. manometer on tilting and that of any of the larger open-end manometers. An example of this derivation of the fraction $\frac{dV}{dP}$ is given in table 1.

With the dogs classed as accurately as possible as immature, young adult, adult and old animals, it was found that the value of the fraction $\frac{dV}{dP}$

varied from 0.132 in an immature dog of 3180 grams body-weight, to 0.272 in an old dog of 14,545 grams. The intradural volume in the same series of animals ranged between 60.8 cc. and 105.1 cc. There was apparently no relationship between spinal length (occipital protuberance to last lumbar spine), body-weight, and intradural volume.

When calculations of the coefficient of elasticity[2] were made by substituting in the formula the values of $\frac{dV}{dP}$ and of intradural volume V for each animal as shown in table 1, it was discovered that the various groups of animals showed an extraordinary constancy in the value of the coefficient

TABLE 1

Derivation of $\frac{dV}{dP}$ and E for dog C-37

MANOMETER	HEAD-DOWN					TAIL-DOWN				
	Pressure-change C.S.F.	Difference in pressure-change	Volume dislocated	Difference in volume dislocated	$\frac{dV}{dP}$	Pressure-change C.S.F.	Difference in pressure-change	Volume dislocated	Difference in volume dislocated	$\frac{dV}{dP}$
mm.	*cm.*	*cm.*	*cc.*	*cc.*		*cm.*	*cm.*	*cc.*	*cc.*	
1	15.4		0.267			8.1		0.141		
4	10.4	5.0	1.009	0.742	0.148	5.6	2.5	0.543	0.402	0.161
6	6.7	8.7	1.769	1.502	0.172	3.3	4.8	0.871	0.730	0.152
8	4.0	11.4	2.040	1.773	0.156	2.1	6.0	1.071	0.930	0.155
10	2.9	12.5	2.067	1.800	0.145	1.5	6.6	1.069	0.928	0.141
Average					0.155					0.152

Average $\frac{dV}{dP} = 0.154$.

Intradural volume = 63.1 cc.

$$E = V \Big/ \frac{dV}{dP} = \frac{63.1}{0.154} = 409 \times 980 \times 1.006 = 4.03 \times 10^5 \text{ dynes per cm.}^2$$

of elasticity. This constancy can be best portrayed in tabular form for the series of 20 animals. In this table 2, the coefficient of elasticity for the immature group is greater in dynes per cm.^{2} than it is in the group of young adults. The adult animals in turn show a somewhat smaller coefficient of elasticity than do those animals graded as young adults, and the obviously old animals of the series show the lowest coefficient of elasticity. In the series portrayed, one dog is obviously out of line with the others of his age-group. This is animal C-36, which was classed as an adult but

[2] This E is a general elastic coefficient for the system as a whole and should not be confused with the coefficient of linear stretch (Young's Modulus) of the membranes and owing to the complexity of the system cannot be directly related to it.

whose coefficient of elasticity would place it in the upper end of the group of young adults. With this exception, the results given in the table for the coefficient of elasticity of the dural sac and its contents exhibit a surprisingly small variation from animal to animal.

Table 2 is compiled from the data obtained only in those experiments where the writers believe that no experimental errors had occurred. Of all the dogs subjected to this experimentation, only three animals were excluded because of such errors. The first of these animals was a small

TABLE 2

Coefficient of elasticity of the dural sac and its contents

EXPERIMENT NO.	WEIGHT	SPINAL LENGTH	INTRADURAL VOLUME	$\frac{dV}{dP}$	ELASTICITY	AGE-GROUP
	grams	*mm.*	*cc.*		*dynes per cm.²*	
C 28	3,180	315	61.9	0.132	4.62×10^5	Immature
C 31	3,250	320	65.0	0.138	4.63×10^5	Immature
C 32	3,440	315	65.1	0.142	4.52×10^5	Immature
C 17	4,520	365	60.8	0.142	4.22×10^5	Young adult
C 20	5,900	390	63.7	0.147	4.26×10^5	Young adult
C 25	7,300	437	67.7	0.152	4.39×10^5	Young adult
C 16	5,074	373	67.0	0.157	4.20×10^5	Young adult
C 18	4,550	350	66.5	0.158	4.15×10^5	Young adult
C 27	4,435	395	70.3	0.163	4.25×10^5	Young adult
C 33	5,350	405	73.0	0.176	4.09×10^5	Young adult
C 35	7,520	435	76.7	0.184	4.16×10^5	Young adult
C 37	8,090	408	63.1	0.154	4.03×10^5	Adult
C 38	4,390	415	72.2	0.177	4.02×10^5	Adult
C 15	7,020	435	73.0	0.178	4.03×10^5	Adult
C 12	4,270	387	75.7	0.186	4.02×10^5	Adult
C 36	13,182	465	88.1	0.197	4.41×10^5	Adult
C 24	9,200	570	94.4	0.230	4.03×10^5	Adult
C 47	8,640	435	73.2	0.188	3.84×10^5	Old
C 45	16,004	482	82.6	0.216	3.78×10^5	Old
C 29	14,545	530	105.1	0.272	3.82×10^5	Old

dog (C-11) in which, because of technical difficulties with the occipital puncture, pressure readings could not be made with accuracy. The second animal (C-19) was excluded because of error in the determination of the intradural volume due to a long tear in the spinal dura mater. The last animal excluded from the table was dog C-21, the findings in which were considered unreliable because of inaccurate determination of the value of the fraction $\frac{dV}{dP}$. With the exception of these three animals, the data from all dogs in the series are included in table 2.

As pointed out in the previous report, an occasional dog showed a marked difference in the value of the fraction $\frac{dV}{dP}$ for the vertical head-down and tail-down tilts. Only one such dog had been found in the previous series, and because of the striking difference in the magnitude of the fraction for the two tiltings the record was included in the previous publication (Weed, Flexner and Clark, 1932) as table 2. In the series of 20 animals shown in table 2 of this report, this phenomenon of difference in magnitude of the fraction $\frac{dV}{dP}$ on head-down and tail-down tiltings was found in 4 animals out of 20. In these animals the head-down values of $\frac{dV}{dP}$ were employed for the determination of the coefficient of elasticity; the tail-down values of $\frac{dV}{dP}$ for these four animals were in general approximately one-half of the head-down values. This finding indicates a difference in elasticity for the two vertical positions in the occasional dog (one out of five). In general, however, the tiltings from the horizontal to the vertical head-down position gave values of the fraction $\frac{dV}{dP}$ quite similar to those obtained on tilting from the horizontal to the vertical tail-down position. Thus, in animal C-27, the head-down tiltings gave a value of 0.162 to the fraction while the contrariwise tiltings yielded a value of 0.165. Again, in animal C-36, the head-down value of $\frac{dV}{dP}$ was 0.197 and the tail-down value 0.196; while in dog C-37, the head down value of $\frac{dV}{dP}$ was 0.155 and the tail-down value 0.152. As a general rule the head-down values of $\frac{dV}{dP}$ were slightly in excess of the tail-down values, but occasionally animals yielded the opposite finding, as in animal C-45, where the head-down tiltings resulted in a value of the fraction $\frac{dV}{dP}$ as 0.201, while the tail-down value was 0.224.

In the determination of the values of $\frac{dV}{dP}$, the four calculations for the head-down tiltings were averaged; and similarly the four calculations for the tail-down tiltings. This procedure resulted in a far more accurate determination of the fraction than any one reading would necessarily give; but when experimental conditions were satisfactory, with no variation in the anesthesia, it was amazing to find how close the individual values of $\frac{dV}{dP}$ would fall (see table 1).

With this determination of the coefficient of elasticity for the various

age-groups of dogs, it becomes possible to calculate the intradural volume for any animal from its individual value for $\frac{dV}{dP}$. Such calculations have been made in table 3 which is composed of data obtained from the same dogs recorded in table 2. The calculated values in most cases differ but slightly from the actual determined values, even in the group of young

TABLE 3

Calculated and measured intradural volumes

The calculation for the intradural contents is made by substituting known values in the formula $E = V \Big/ \frac{dV}{dP}$ or $V = E \frac{dV}{dP}$. In this substitution dP = height in centimeters × acceleration of gravity (980) × density (1.006).

EXPERIMENT NO.	AVERAGE ELASTICITY	$\frac{dV}{dP}$	CALCULATED INTRADURAL VOLUME	MEASURED INTRADURAL VOLUME	DIFFERENCE FROM CALCULATED VOLUME	AGE-GROUP
	dynes per cm.²		*cc.*	*cc.*	*cc.*	
C 28	4.58×10^5	0.132	61.4	61.9	+0.5	Immature
C 31	4.58×10^5	0.138	64.2	65.0	+0.8	Immature
C 32	4.58×10^5	0.142	66.0	65.1	−0.9	Immature
C 17	4.22×10^5	0.142	60.8	60.8	0	Young adult
C 20	4.22×10^5	0.147	62.9	63.7	+0.8	Young adult
C 25	4.22×10^5	0.152	65.1	67.7	+2.6	Young adult
C 16	4.22×10^5	0.157	67.2	67.0	−0.2	Young adult
C 18	4.22×10^5	0.158	67.6	66.5	−1.1	Young adult
C 27	4.22×10^5	0.163	69.8	70.3	+0.5	Young adult
C 33	4.22×10^5	0.176	75.3	73.0	−2.3	Young adult
C 35	4.22×10^5	0.184	78.7	76.7	−2.0	Young adult
C 37	4.03×10^5	0.154	63.0	63.1	+0.1	Adult
C 38	4.03×10^5	0.177	72.4	72.2	−0.2	Adult
C 15	4.03×10^5	0.178	72.8	73.0	+0.2	Adult
C 12	4.03×10^5	0.186	76.1	75.7	−0.4	Adult
C 36	4.03×10^5	0.197	80.6	88.1	+7.5	Adult
C 24	4.03×10^5	0.230	94.1	94.4	+0.3	Adult
C 47	3.81×10^5	0.188	72.7	73.2	+0.5	Old
C 45	3.81×10^5	0.216	83.6	82.6	−1.0	Old
C 29	3.81×10^5	0.272	105.3	105.1	−0.2	Old

adult dogs where the maximum difference between the calculated and measured values is 2.6 cc. in experiment C-25; this maximum variation is approximately 4 per cent. In the group of adult dogs where the average is based on five animals whose coefficients of elasticity were either 4.02 or 4.03×10^5 dynes per cm.², the one animal out of line (C-36) showed a difference between calculated and determined values of the intradural contents of 7.5 cc., an error of less than 10 per cent.

Other types of calculation may similarly be made by substitution in the general formula for the coefficient of elasticity. If the total volume of the intradural contents is known, it is possible to calculate the value of the fraction $\frac{dV}{dP}$ on the basis of the average coefficient of elasticity for that age-group. Quite similarly, if the value of the fraction $\frac{dV}{dP}$ is determined for any animal or for those of similar size and age (where the volume V may be assumed to be the same), any change in elasticity may easily be ascertained. Likewise, if the value of $\frac{dV}{dP}$ is known for any one animal or group of the same size and age, any change in the pressure of the cerebrospinal fluid (dP) will permit a calculation of the volume-change (dV) of the intradural contents (as when the nervous system is decreased in volume by dehydration).

Discussion. The data presented in the foregoing pages indicate that it is possible to obtain a coefficient of elasticity of the cerebrospinal system of living animals by application of the usual physical formula for such determinations. Within the various age-groups of dogs, the coefficient of elasticity was found under satisfactory experimental conditions to be of considerable constancy, the immature animals giving values of approximately 4.6×10^5 dynes per cm.^{2}, while the old animals yielded values in the neighborhood of 3.8×10^5 dynes per cm.^{2} Between these two limits occurred the coefficients of elasticity of the groups of animals classed as young adults and adults. The reason for the apparent separation of the values of the two groups at the extremes of the classification lies in the fact that an attempt was made to secure for these two groups a few animals obviously immature though of suitable weight, and a few animals obviously old. Were the series of dogs to be even more widely extended, covering animals of all ages from birth to senility, the values of the coefficient of elasticity would undoubtedly merge gradually from one group into the other.

It seems very remarkable that a physical formula used for the determination of the coefficient of elasticity of solids, fluids, and gases on compression, could be applied with profit to the problem of meningeal elasticity so that the relationship of the external dislocation of cerebrospinal fluid to its resultant pressure might be expressed as a fraction $\frac{dV}{dP}$ with a constant value for any one animal. In the case of the central nervous system, it is obvious that we are not dealing with the phenomenon of compression of the contents of the intradural cavity nor with the possible dilatation of these contents under conditions of reduced pressure. Rather are we here dealing with the elasticity of the membranes and vascular bed

which permits an internal shift of fluid from one part of the nervous system to another and an external dislocation into or from manometers under conditions of positional change. In the experiments under discussion the total volume of the intradural contents may be taken to vary only to the extent of the amount of dislocation of fluid into or from the manometer as any intradural volume-change of the cerebrospinal fluid is at least in part compensated for by reciprocal variation in the total amount of blood within the dural sac. This reciprocal compensation in the blood vascular volume cannot be complete or the total volume V would not vary by the amount dV and the formula for elasticity could not be applied. But by means of the reciprocal compensation in the vascular bed, the internal readjustment in volume is kept constant enough, throughout the range of the experiments, to insure a constant relationship between external volume and pressure-changes. Such a statement of course implies a constancy in the volume of the true central nervous tissue itself (brain and spinal cord), but under the conditions of experimentation these two structures may be taken to be of unchanging volume.

In addition to such reciprocal compensation as may occur within the blood vessels of the meninges and central nervous system, there is a strong likelihood of somewhat analogous compensatory phenomena occurring within the venous plexus of the epidural space. Such changes in these thin-walled vessels would play but little rôle when the spinal dura is put under additional pressure as in the tail-down tilts, but in the contrariwise positional tiltings the dilatation of this extensive venous bed would permit an inward collapse of the spinal dural tube such as would not be possible without an elastic element within the epidural space.

While emphasis has been placed upon the dislocation of cerebrospinal fluid in the pressure-changes ensuing upon abrupt positional change in these dogs, it is realized that this fluid is merely a medium in affording a means of pressure-change and volume-change. The pressure-change dP, while existing in the fluid itself, is actually reflected by the fluid upon all of the intradural structures, so that all parts of this cerebrospinal system are affected in some way by the recorded pressure-change. Somewhat similarly the volume-change dV represents not an alteration in the total volume of the cerebrospinal fluid (though in the experiments the calculation of dV is made solely upon the fluid) but an alteration in the total volume of the contents of the dural envelope (cranial and spinal), whether of nervous tissue itself, of blood vascular bed, or of the cerebrospinal fluid. Because of these considerations the total volume of the intradural contents has been taken to represent the volume V in the formula used.

Attention has been given to the fact that throughout the period of experimentation there must be a continuous production and absorption of cerebrospinal fluid. This production of fluid, with its equivalent absorp-

tion, does not seem to modify the relationship between the dislocation of fluid and its pressure, even though the pressure-reactions suggest that the absorption of fluid is more rapid in the head-down position than in the horizontal and tail-down positions. Any difficulties in this regard are apparently eliminated by our practice of determining the pressure-changes in the cerebrospinal fluid with reference to the pressure in the horizontal position before tilting rather than to the pressure recorded on return to the position (cf. Weed, 1929a, b). Uniform procedures followed for all the manometers would yield comparable results and would eliminate any possible errors from these factors of production and absorption.

The question naturally arises as to the exact meaning of the term coefficient of elasticity, when applied to such a complex organization as that of the cranio-vertebral contents. Elasticity, to accept the physicists' definition, is power to resist change in shape, or power to resume the original shape after deformation. It follows therefore that, under a given change in pressure, the greater the change in volume, the smaller is the coefficient of elasticity. The high values in dynes, obtained for the coefficient of elasticity of the cerebrospinal system in immature animals, indicate that more pressure is required to effect a given change than is required for the old animals of the series. This means that in these immature animals the power of the cerebrospinal system to resist deformation of the contents (or as determined in these experiments, dislocation of cerebrospinal fluid) is greater than in the group of fully adult and old animals. This general finding would seem, at first glance, to be in direct opposition to one's preconceived ideas that the younger animals should have smaller coefficients of elasticity than the older. Were elasticity in the physicists' sense merely a matter of stretch or distensibility, these findings would seem to be erroneous, but the power to resist deformation appears here to be a complex function of the cerebrospinal system, compounded of vascular factors (systemic vasomotor tone, intradural vascular readjustment, etc.) as well as the inherent distensibility and collapsibility of the dural sac. And it must be emphasized that the elasticity of this latter anatomical mechanism is largely related to the method and character of the suspension of the spinal dural envelope in the vertebral canal. The possibility of a greater inward collapse of the spinal dura in animals of advancing age does not seem too remote for consideration. There is at hand no definite knowledge of the relative size of the epidural space in dogs of different ages; nor is it known whether the fibrous trabeculae of this space increase with age rather than diminish in functional importance due to increasing depositions of fat. If, as repeated inspection indicates, the volume of this epidural tissue is relatively greater in adult and old animals than in immature, the inward collapse of the spinal dural tube in old animals might be far in excess of that in young. Again, there is a strong possibility that

the relative intradural volumes of blood, of brain and spinal cord, and of cerebrospinal fluid undergo definite age-changes; here also we are confronted with impressions rather than established anatomical facts for the dog. A relative decrease in the fixed tissues of the intradural space in advancing age would increase the mobile elements relatively and therefore change the coefficient of elasticity. Such explanations of the general phenomenon of the difference in cerebrospinal elasticities in animals of different ages have much in their favor as a hypothetical consideration: they lay greater stress upon the anatomical factors than upon the purely physiological ones of vascular tone. It may be however that the more important age-change occurs in the vascular system, but such age-changes as are known physiologically in this system are those of the general systemic circulation rather than of the intradural.

The exact determination of the coefficient of elasticity of the cerebrospinal system in dynes per square centimeter permits the introduction of a definite component of elasticity into current conceptions of the degree of rigidity imposed upon the central nervous system by the bony coverings of cranium and vertebral arches. The Monro-Kellie hypothesis may now be restated with very definite knowledge of the elastic factor of the craniovertebral contents (Weed and Flexner, 1932).

SUMMARY

In dogs of different sizes, weights and ages, the relationship between the dislocation of the cerebrospinal fluid and its pressure, expressed in the fraction $\frac{dV}{dP}$, has been determined by tilting the animals from the horizontal to the two vertical positions. The total volume V of the intradural contents (cranial and spinal) has also been measured. These data have been substituted into the physical formula for the determination of the coefficient of elasticity $E = V\Big/\frac{dV}{dP}$. The coefficient of elasticity has been found to have the following values in dogs:—immature, 4.63 to 4.52 $\times$ 10^5 dynes per cm.^{2}; young adult, 4.39 to 4.09 $\times$ 10^5 dynes per cm.^{2}; adult, 4.03 to 4.02 $\times$ 10^5 dynes per cm.^{2}; old, 3.84 to 3.78 $\times$ 10^5 dynes per cm.^{2}

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OBSERVATIONS ON THE CIRCULATION IN THE FETAL ALBINO RAT

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Hartman, Squier and Tinkelpaugh (1930) have reported that the fetal heart rate of the monkey (*Macacus rhesus*) is lower than that of the mother. They consider the macaque to be exceptional in this respect. The observations of Clark (1927) indicate that in man, the cow and the dog the fetal heart rate is roughly double that of the mother. Williams (1913) states that the human fetal heart rate is between 120–140, while the average maternal rate is 70 beats per minute. In a summary of 300 cases, Corfield (1930) agrees with the findings of Williams.

The idea that the female fetal heart rate is uniformly higher than that of the male is denied by both Williams and Corfield. Observers appear to be in agreement that a high degree of variation in the fetal heart rate is present. Hartman and his co-workers state that the fetal heart rate of the monkey is "unpredictable."

The factor or factors governing the fetal heart rate remain as obscure as ever. Rech (1931a, b) in two brief papers, has brought forth evidence that no correlation exists between the blood gas content of the fetal and maternal blood. Kellogg (1930) reports very little difference in the carbon-dioxide content of the blood of very young and term fetuses. From the above, the desirability of studying cardiac activity in an extended series of fetuses throughout a considerable portion of the gestation period seemed evident.

The albino rat was selected for study because of its high fecundity and ease of handling. Although the rat fetus is small, a fairly wide experience with this animal (Corey, 1932) provided convincing evidence that it could be profitably utilized. The heart rate of 138 fetuses comprising 21 litters was ascertained. The blood velocity in 62 fetuses (13 litters) and the blood pressure of 16 fetuses (4 litters) were also determined. The fetuses measured from 8 to 39 mm. in crown-rump length, and represented the latter half of the gestation period.

The pregnant rat was lightly anesthetized with ether or amytal and placed on a water-jacketted operating table. The body wall was incised and one fold of the uterus drawn out on the laparotomy cloth. The uterus

and sac were then incised and the fetus observed. A minimum of stimulation to mother and fetus was produced. In recording the maternal heart rate, initial and final observations were made. Both palpation and direct observation of the heart were employed. Very little variation in the maternal heart rate was observed throughout the period of experimentation.

In very young fetuses it was found possible to observe the beating heart through the body wall; in the older fetuses, however, this was more difficult and all observations were checked by opening the thorax (with practically no hemorrhage) and observing the heart directly. The rates recorded by the use of both methods were found to be fairly constant, and no significant variation was observed in fetuses maintained in saline solution at 37°C. in contrast to those operated in air.

In computing the approximate fetal blood velocity, India (carbon) ink was introduced into the heart by means of a fine hypodermic needle, specially constructed. The needle was attached to a $\frac{1}{4}$ cc. syringe. The time elapsing between the admission of the ink and its appearance in the umbilical artery was recorded by use of a stop watch. Subsequent clearing of the fetuses and measurement of the vessels traversed by the ink yielded the approximate blood velocity. While the possible errors in the method are apparent, the results obtained may be considered to be comparable. Great care in manipulation was necessary, since clogging of the vessels, excessive pressure on the plunger and other factors often lead to poor results. Only in cases in which the manipulation was considered to be adequately uniform were the results tabulated.

Blood pressure determinations were made by means of a specially constructed manometer, involving the pressure of a thin rubber membrane on the umbilical cord to produce occlusion of the artery. On release of pressure, the influx of blood to the exsanguinated area was used as a criterion of the fetal systolic pressure. The results obtained are summarized in table 1. Figure 1 shows the results graphically.

The fetal heart fluctuates greatly in rate throughout the latter half of the gestation period. Three periods of greatest activity are apparent: at the 16.4, 23.3 and the 34.2 mm. stages. At term the fetal heart rate is slightly slower.[1] Considerable variation in heart rate may occur amongst members of the same litter: this difference amounted to 92 beats per minute in litter 24. There is no evidence of synchronous heart action among the members of a litter.

The average heart rate of male fetuses exceeds that of females in all litters observed, with but one exception (no. 13). Individual females may have heart rates greatly in excess of males of the same litter. No

[1] This is in agreement with the results reported by Hartman *et al.* on Macacus.

marked sex difference in heart rate is therefore apparent. The fact cannot be overlooked, however, that in all but one litter the male heart rate

TABLE I

LITTER NUMBER	AVERAGE CROWN-RUMP LENGTH	NUMBER OF FETUSES IN UTERO	MATERNAL HEART RATE (BEATS PER MINUTE)	AVERAGE FETAL HEART RATE (BEATS PER MINUTE)	FETAL HEART RATE (EXTREMES)	AVERAGE HEART RATE (MALES)	AVERAGE HEART RATE (FEMALES)	AVERAGE BLOOD VELOCITY
	mm.							*mm. per second*
7	8.0	5	184	117	96–114			
11	15.3	8	248	184	168–196	185	180	
8	16.4	5	228	197	192–200	198	196	
3	18.8	9	216	159	136–184	160	158	
5	20.2	4	212	169	164–172	170	168	
22	20.9	10						11.1
10	21.8	7	252	213	192–223	214	210	
19	22.7	11	248	156	144–216	162	151	23.3
9	23.3	7	280	223	212–232	226	221	
6	24.0	5	228	185	164–192	192	180	
17	25.8	7	228	198	188–212	204	192	15.0
16	26.4	7	240	195	184–212	212	186	8.0
1	27.2	4	212	138	123–143	143	133	
23	27.3	8						4.7
21	31.7	8						10.8
14	32.6	8	256	189	176–200	190	188	13.8
12	34.2	7	264	243	224–256	246	240	
13	34.8	6	250	206	196–216	206	206	10.3
24	35.0	8	224	159	128–210	169	148	10.0
20	35.2	7						14.2
25	35.4	8	216	207	184–248	211	203	26.1
15	35.6	8	208	208	204–212	212	204	31.8
18	36.0	6	228	191	160–216	202	185	21.8
2	38.6	3	238	219	208–232	232	212	
4	39.2	5	228	185	172–196	188	180	

Total number of fetuses observed: 171.
Average blood velocity: 15 mm. per second.
Average blood pressure: 10 mm. Hg.
Average maternal heart rate: 223 beats per minute.
Average fetal heart rate: 187 beats per minute.
Average heart rate for male fetuses: 195 beats per minute.
Average heart rate for female fetuses: 187 beats per minute.
Blood pressure readings were made from litters 8, 9 and 10.
The anesthetic used on each animal has been omitted from the table since similar results were obtained with the use of either amytal or ether.

averaged somewhat higher. This finding can hardly be attributable to coincidence.

A distinct parallelism between the maternal and fetal heart rates is

apparent (see fig. 1). The fetal heart rate is observed to rise and fall, in the majority of cases, in approximate correlation with that of the mother. Vagal stimulation of the maternal heart was performed in litters 8, 9 and 10. No observable change took place in the heart rate of the fetuses *in utero*. No explanation of this maternal-fetal correlation in heart rate is offered at this time.

Checks on blood velocity and blood pressure roughly corresponded to the above observations. While the inaccuracies involved in obtaining the blood velocity are appreciated, the results may nevertheless be considered as comparable. Both blood velocity and pressure determinations parallel approximately the heart rate curve. The latter can be taken as a

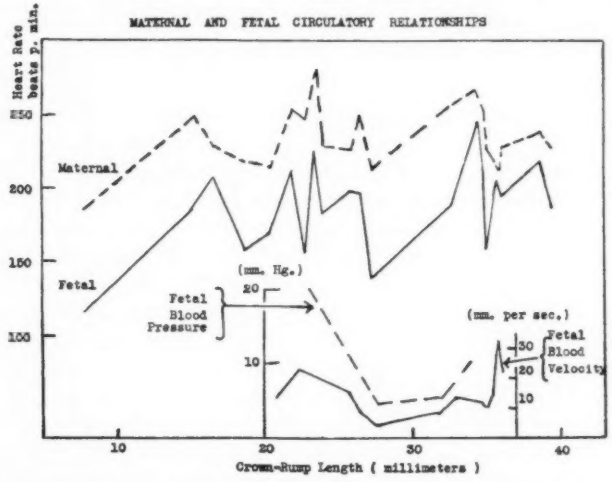

Fig. 1. Showing maternal and fetal heart rates (above); fetal blood velocity and blood pressure (inset below) during the latter half of the gestation period.

fair representation of cardiac activity throughout the latter half of the gestation period.

SUMMARY

The heart rate, blood velocity and blood pressure during the latter half of the gestation period have been determined in the albino rat fetus.

The fetal heart rate is lower than that of the mother. There is an apparent correlation between the heart rate of the mother and that of the fetuses in utero.

The heart rate does not increase regularly with advancing fetal development; considerable variation is observed. Three major rhythms in cardiac activity were noted during the portion of the gestation period studied.

There appeared to be a slight sex difference in heart rate in favor of the male.

Curves showing the blood velocity and blood pressure parallel approximately that representing the heart rate.

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THE EFFECT OF CASTRATION IN THE GUINEA PIG UPON THE SEX-MATURING POTENCY OF THE ANTERIOR PITUITARY[1]

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The dependence of the gonads upon the anterior pituitary for their normal development is established, and likewise the fact that gonadectomy results in striking cellular changes in the hypophysis. Engle (1929) added to an understanding of this pituitary-gonadal relationship by implanting the anterior lobes of castrated rats into immature mice and rats. He demonstrated in a series of 31 animals that the ovarian response of immature mice and rats to fresh transplants of anterior pituitary from castrated rats was far greater than that obtained with similar transplants from normal animals. These results were substantiated by Evans and Simpson (1929). Such findings are of particular interest because of the cytological changes which Addison (1917) and others had described in the pituitary of the castrate rat. These changes consist largely in an increase in the number of large basophiles, and in the appearance of vacuolated "signet ring" cells which have been called the "castration cells." Engle related the increased gonad-stimulating potency of the castrate's pituitary to the presence of these basophilic castration cells, being "led to suspect that it is the basophile cell which elaborates the gonad stimulating factor in the rat," and that the castration cells represent storage of the hormone.

An examination of the literature indicated, however, that in most of the laboratory mammals, gonadectomy results in an increase of acidophilic cells in the pituitary. Fichera (1904) first described such an acidophilic increase in the guinea pig, and he was confirmed by Kolde (1912) and others. In the light of previous experiments on the rat, where the cytological picture is in such contrast, it was of interest to test the gonad-stimulating influence of the anterior pituitary of the castrated guinea pig against that of the normal. The present paper deals with such experiments.

EXPERIMENTAL. A series of 60 guinea pigs, half males and half females, constituted the pituitary donors. Fifteen males and fifteen females were

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gonadectomized, and a similar number in each sex kept as controls. The guinea pigs were all of one inbred stock, and gonadectomy was performed at the approximate age of 30 days when their body weights ranged around 300 grams. The gonadectomized animals were sacrificed at intervals ranging from 72–205 days. Their body weights as well as those of the controls sacrificed with them were carefully recorded. In the normal females the stage in the oestral cycle was determined by daily observations and at sacrifice was recorded in the data as the number of days following the closing of the vagina.

The recipients of the transplants were littermate female white mice from remarkably uniform stock supplied through the courtesy of Dr. E. C. MacDowell of Cold Spring Harbor. Three or four littermate females constituted an experimental series in which one mouse received an intramuscular implant of a whole anterior lobe from a castrated guinea pig, one mouse a whole gland from a normal guinea pig of like sex and age, and one or two mice remained as controls. In series 8 to 11, two implants of $\frac{1}{2}$ gland on consecutive days were used in place of a single implant of a whole gland. The implants were made by the Smith method. The recipients were 20 day old mice, except in series I and II in which the mice were 22 days of age. The mice were examined on the second day following the transplant for opening of the vagina and sacrificed for autopsy on the third day following (age 23 days).

At autopsy the ovaries and the uteri were weighed and their appearance under the binocular microscope noted. All material was fixed in Bouin, sectioned at 8μ and stained in Harris hematoxylin and eosin. Engorged uteri were always drained of their fluid content before weighing to meet the legitimate criticism of Engle (1927, p. 104) that a large part of the variation in uterine weight consisted of a transitory fluid content. Several guinea pig pituitaries both of castrated and normal males and females at ages corresponding to the implant series were preserved for cytological study according to a method previously described (Severinghaus, 1932). Data are presented in composite form in table 1.

Ovarian and uterine response to transplanted pituitaries. The gonad-stimulating potency of the pituitary transplant is measured by the weight and development of the immature mouse ovaries. The greater the weight and development, the greater the amount of gonad-stimulating hormone contained in the transplanted pituitary. An analysis of the data shows that pituitaries from castrate guinea pigs are able to effect uniformly a greater growth response in the ovaries of recipient immature mice than is produced by similar implants of pituitaries of normal guinea pigs. This is true both for male and female donors, and in the females regardless of the stage in the oestrous cycle of the controls. The average weight of the ovaries following transplants of anterior lobe from castrated animals is

TABLE 1

Gonadal-stimulating action of anterior pituitary from normal and castrated guinea pigs

SERIES	NORMAL AND CASTRATED GUINEA PIG DONORS					RECIPIENT IMMATURE MICE					
	Number of animal	Sex	Days castrated	Weights		Number of animal	Treated at		Autopsy		
				At age 1 mo.: time of castrations	When sacrificed		Age	Weight	Age	Weights	
										Ovaries	Uterus
				grams	*grams*		*days*	*grams*		*grams*	*grams*
I	11	♀	183	331	680	34	22	7.05	25	0.0054	0.0294
	22	♀	0	320	502	33	22	7.10	25	0.0041	0.0215
						32		7.27	25	0.0029	0.0052
						35		6.85	25	0.0028	0.0067
II	12	♀	185	320	760	47	22	8.10	25	0.0067	0.0415
	20	♀	0	385	620	46	22	7.70	25	0.0078	0.0437
						45		8.80	25	0.0025	0.0091
III	15	♀	185	295	550	91539	20	10.75	23	0.0083	0.0355
	18	♀	0	285	595	91538	20	10.90	23	0.0058	0.0461
						91537		11.77	23	0.0046	0.0120
IV	16	♀	185	362	590	91542	20	9.62	23	0.0116	0.0444
	21	♀	0	358	455	91543	20	9.68	23	0.0057	0.0509
						91541		10.15	23	0.0040	0.0127
V	1	♂	186	307	590	91647	20	9.35	23	0.0074	0.0523
	28	♂	0	350	530	91545	20	9.52	23	0.0048	0.0372
						91546		9.77	23	0.0036	0.0115
						91548		8.74	23	0.0037	0.0087
VI	2	♂	205	336	740	92324	20	8.94	23	0.0118	0.0527
	30	♂	0	400	810	92323	20	9.05	23	0.0042	0.0520
						92326		8.70	23	0.0038	0.0084
						92325		9.45	23	0.0034	0.0110
VII	3	♂	205	402	810	92320	20	9.29	23	0.0079	0.0485
	29	♂	0	390	790	92322	20	9.33	23	0.0046	0.0465
						92321		9.79	23	0.0036	0.0086
						92319		9.09	23	0.0037	0.0081
VIII*	31	♂	104	350	610	92308	20	9.22	23	0.0095	0.0442
	38	♂	103	340	590		21				
	39	♂	0	?	660	92306	20	11.19	23	0.0058	0.0475
	26	♂	0	275	440		21				
						92309		11.75	23	0.0025	0.0080

* In series VIII–XI inclusive two implants of ½ gland each were given on consecutive days.

TABLE 1—*Concluded*

SERIES	NORMAL AND CASTRATED GUINEA PIG DONORS					RECIPIENT IMMATURE MICE				
	Number of animal	Sex	Days castrated	Weights		Number of animal	Treated at		Autopsy	
				At age 1 mo.; time of castrations	When sacrificed		Age	Weight	Age	Weights
										Ovaries / Uterus
				grams	*grams*		*days*	*grams*		*grams* / *grams*
IX	33	♂	104	390	600	92318	20		23	0.0110 / 0.0412
	7	♂	206	378	550		21			
	40	♂	0	?	540	92313	20		23	0.0056 / 0.0550
	9	♂	0	430	860		21			
						92315			23	0.0039 / 0.0135
X	48	♀	78	405	505	92412	20	10.94	23	0.0088 / 0.0615
	60	♀	72	408	440		21			
	50	♀	0	270	340	92411	20	10.99	23	0.0054 / 0.0270
	56	♀	0	405	465		21			
						92413		9.95	23	0.0038 / 0.0093
						92410		11.23	23	0.0042 / 0.0123
XI	48	♀	78	405	505	92401	20	10.39	23	0.0097 / 0.0482
	60	♀	72	408	440		21			
	50	♀	0	270	340	92398	20	10.98	23	0.0054 / 0.0520
	56	♀	0	405	465		21			
						92397		10.38	23	0.0040 / 0.0156
						92399		11.17	23	0.0051 / 0.0115
XII	8	♂	206	356	600	92402	20	9.02	23	0.0051 / 0.0465
	10	♂	0	385	890	92403	20	9.22	23	0.0068 / 0.0463
						92404		9.74	23	0.0036 / 0.0102
XIII**	a	♀	4½ mo.			M1379	20	9	23	0.0084 / 0.0433
	C111	♀	0			M1380	20	9	23	0.0032 / 0.0263
	C149	♀	0			M1378	20	9	23	0.0033 / 0.0143
XIV	b	♀	4½ mo.			M1376	20	11	23	0.0068 / 0.0477
	C115	♀	0			M1369	20	10	23	0.0054 / 0.0324
	C165	♀	0			M1377	20	12	23	0.0041 / 0.0300
	C107	♀	0			M1359	20	12	23	0.0036 / 0.0120

** Series XIII, XIV are from previously unpublished data of Smith and Engle.

0.0085 gram while that from normals is 0.0050 gram. The control non-implanted mice show a total ovarian weight of 0.0036 gram. These weight increases, while significant, are not so great as those reported by Engle for the rat.

The gross appearance of the ovaries is also of interest. Almost inva-

riably the ovaries which had been stimulated by pituitaries of castrates showed clearly visible follicles, often presenting a surface of berrylike appearance. Although small follicles were seen occasionally in ovaries in response to an implant from a normal guinea pig, not infrequently the enlarged ovary showed externally no distinct follicles.

Microscopic examination of the ovaries is more instructive. Those which have been subjected to the influence of an implanted pituitary from a castrated guinea pig present a picture of mature development. A row of fully formed follicles makes up the bulging peripheral contour of the gland, and is responsible for its gross "berry" appearance. The increased size is obviously due to this large number of matured follicles. The maturing response of the ovary to a pituitary implant from a normal guinea pig is comparable to that just described, but is a response of lesser degree. This is especially true as regards the number of follicles which have been influenced to develop; and of this smaller number, many do not depict the complete growth so characteristic of follicles acted upon by an implant from a castrate's pituitary. The ovarian responses described above are in all respects similar to those reported in the mouse by implants of pituitaries from other species (Smith and Engle).

It is of interest to note that uterine weight increases are not always proportional to the increases in ovarian weight. In several instances a slightly enlarged ovary, following a pituitary implant from a normal guina pig, was associated with a greatly enlarged and engorged uterus. Microscopic examination of these cases showed the presence of a very few greatly enlarged follicles in an otherwise immature ovary. A similar condition was described after implants of cat pituitary (Smith and Engle, 1927, fig. 27). Obviously the introduced hormone is of such nature and amount as to influence only a few follicles which are of proper physiological state, and it does not call forth the more general growth and maturing response of follicles which larger amounts of hormone are capable of. These few follicles, however, are sufficient to cause marked changes in the uterus. The uterine response is thus the result of a few activated follicles, although the ovary as a whole may have changed very little in size, weight or development.

In series II the implant from guinea pig 20 deserves, perhaps, a word of comment. It will be noticed that the pituitary implant from the normal female has produced in this instance a slightly larger ovarian response than is shown by the littermate sister which received the implant from a castrated guinea pig. This exception to the otherwise uniform data is interesting in view of the fact that the normal donor had been observed to have irregular cycles. The uterine weight was about one-half that of normals. It is quite possible that some ovarian pathology resulted in changes in the pituitary which increased its potency. The ovaries did not appear cystic, but, unfortunately, were not preserved for microscopical study.

DISCUSSION. The rather uniform data lead unmistakably to the conclusion that the anterior pituitary of the castrated guinea pig contains more sex-maturing hormone than does the pituitary of the normal control. These results are in agreement with the findings of Smith and Engle (unpublished but here included) in a smaller series of guinea pigs. They are of particular significance because of the great cytological difference between the anterior pituitary of the castrate rat and the castrate guinea pig. In the rat, as has been pointed out, castration results in a marked increase of large basophiles and in the appearance of numerous vacuolated "castration cells" which are unmistakably modified basophiles. That these basophiles are the source of the gonad-stimulating hormone in the rat and that the castration cells represent its storage, a supposition made by Engle, seems almost irresistible. Thus the cytological findings meet all the requirements of an explanation for the increased potency of the castrate rat pituitary.

The transplantation of the castrate guinea pig pituitary has complicated the explanation. In this gland we have a similar though less marked increase in potency after gonadectomy, but no noticeable increase in the basophilic cells and a conspicuous absence of the typical "castration cell." Although the counting of a large series of animals is necessary to determine cell type proportions accurately and to detect any deviation from the normal, a survey of serial sections through the pituitaries of a few castrate guinea pigs is sufficient to confirm previous investigators that a noticeable increase in basophiles does not occur, and, so far as the writer is concerned, to leave one unwilling to deny the assertion that an actual increase of acidophiles has taken place. Such an increase could certainly not be established by routine examination of a few animals. A more detailed cytological study of the pituitary must be left for another time. What cells, then, in the anterior lobe of the castrate guinea pig are responsible for the increased amount of gonad-stimulating hormone? No answer seems convincing at this time. It becomes necessary, however, in view of these results to avoid any generalization concerning the specific cells responsible for the sex-maturing hormone. We are again faced with the necessity of admitting species differences to our interpretations. What is so obviously the case in the rat cannot possibly be applied to the guinea pig. Although these experiments do not further us directly in our attempt to localize the cells of origin of the gonad-stimulating hormone, they are valuable in reminding us again that results obtained in experiments on one species cannot forthwith be applied to all forms.

It is a pleasure to acknowledge the invaluable assistance of Dr. P. E. Smith in these studies, especially in the autopsies of the implanted mice.

SUMMARY

1. The ovarian response of immature mice following the intramuscular transplantation of one anterior pituitary from a gonadectomized guinea pig of either sex is significantly greater than the response to a similar transplant from a normal animal.

2. Although the series of transplantations is small, it indicates no sex-difference in the potency of the gland in either castrates or normals.

3. Glands from castrates of 72 days' duration and those of 206 days did not differ significantly in potency.

4. Inasmuch as the basophiles do not increase following castration in the guinea pig and the typical "castration cells" are missing, these two cell types cannot be designated respectively as the source and storage of the increased gonad-stimulating hormone.

5. These experiments compel caution in the generalization of data, and emphasize the importance of species differences in the interpretation of endocrine phenomena.

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THE END OF THE SPIKE POTENTIAL OF NERVE AND ITS RELATION TO THE BEGINNING OF THE AFTER-POTENTIAL

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The least known part of the action potential of nerve is that immediately at the end of the spike. The form of the spike has been well worked out up to the time when its potential, having fallen to a few percent of its maximum value, becomes fused with the after-potential. As the diphasic artifact causes its most disturbing distortion at this time, the region has been very resistant to further analysis. A clue which promised a solution appeared in some experiments on veratrinized nerve (9). When such a nerve was stimulated there appeared, just after the diphasic artifact, a second trough (as in fig. 3, *1* in the present paper) which in turn was followed by the after-potential, the latter not reaching its maximum until somewhat later. The conditions of the experiment were such that there was no possibility that the crest following the diphasic artifact was a delayed wave, and the only reasonable solution which presented itself was that all the negativity subsequent to the diphasic artifact was not after-potential as had been assumed, but that a portion of it belonged to the end of the spike.

The little trough in the early part of the after-potential had to have a name for reference purposes, and we gave it the purely descriptive one of N_2, or second notch, the first notch being the diphasic artifact. When N_2 had once obtruded itself upon our attention in veratrinized nerves, a search for it in normal nerve and in nerves otherwise treated showed that it could generally be identified in the same position, though its size was such as to cause it to be easily overlooked or dismissed as an artifact. In form it was usually not a notch at all, but merely the locus of a more rapid change in the slope of the line. The constancy of the phenomenon indicated that it must have a meaning, and the experiments reported in this paper were designed to test the hypothesis that it is occasioned by the end of the spike.

That there should be in a "monophasic" lead a remnant of the spike after the apparent end of the diphasic artifact is at first sight surprising

but a theoretical reconstruction soon shows that it is a natural consequence of the form of the spike.

The monophasic spike. For reconstruction purposes it is necessary to employ a form of the spike in which there is little distortion at the end on account of diphasicity. Previous methods of approximating this have been to locate the diphasic artifact directly under the first phase as the result of a very short interpolar distance (6), or to place the stimulus near the second lead so that the front rather than the tail of the wave is distorted (Bishop, 1927). Recently another method has become available as the result of a chance observation. It was noticed that nerves, which had been heat coagulated at the end for a monophasic lead, lost their residual diphasicity when the whole nerve was painted with Ringer's solution rich in potassium. This suggested that a nerve treated with potassium at one lead would be more monophasic than one prepared by crushing or heating, and such proved to be the case. The only difficulty is the danger of spread of the potassium along the nerve to the active lead, an event easily leading to erroneous conclusions in after-potential experiments since potassium decreases the after-potential more than it does the spike (8).

Another desirable quality in the spike form used for reconstruction purposes is freedom from after-potential, but no means of obtaining this is available: the schemes for reducing the after-potential are only differential with respect to the spike. The after-potential can be held to a small value, however, if a nerve be used fresh and kept at a cool temperature. The spike used in the following reconstruction and reproduced in figure 1 (solid line) was obtained at 14.2° from fresh frog nerve made monophasic with potassium. The experiment was performed under conditions eliminating temporal dispersion and the final form was synthesized from records made at two amplifications, one for the form of the early high portion and the other for the decremental tail. The observed crest time was 0.82σ; but inasmuch as at the ordinary experimental temperature 0.3σ is the usual figure the spike was calculated to this value. This procedure is justifiable because the relative form is not altered by temperature changes (6).

Derivation of the diphasic spike from the monophasic. The graphic derivation of the theoretical form of a diphasic response is shown in figure 1. The lower part of the monophasic spike described in the preceding paragraph is plotted above the zero line. On the assumption that the second phase is 10 per cent of the first and starts 0.5σ after it, the same spike calculated to the designated relative size is plotted in the appropriate position and in the reverse direction below the line. The latter curve gives the complete form of the spike as obtained in the experiment. The algebraic sum of the two curves—shown as an interrupted line—gives the form of the corresponding diphasic spike.

In actual experiments the curves obtained may vary considerably from the one shown in the figure because of other conditions. The relative magnitude to which the artifact is manifest will be altered by the interpolar distance and by temporal dispersion of the second phase. The theoretical curve is derived for a rather long interpolar distance; distances longer or shorter than this would make the second phase more or less apparent. Temporal dispersion, which is inevitable in the second phase even if all the impulses start from the first lead simultaneously, will make the second phase more apparent by causing it to be recorded under a lower part of

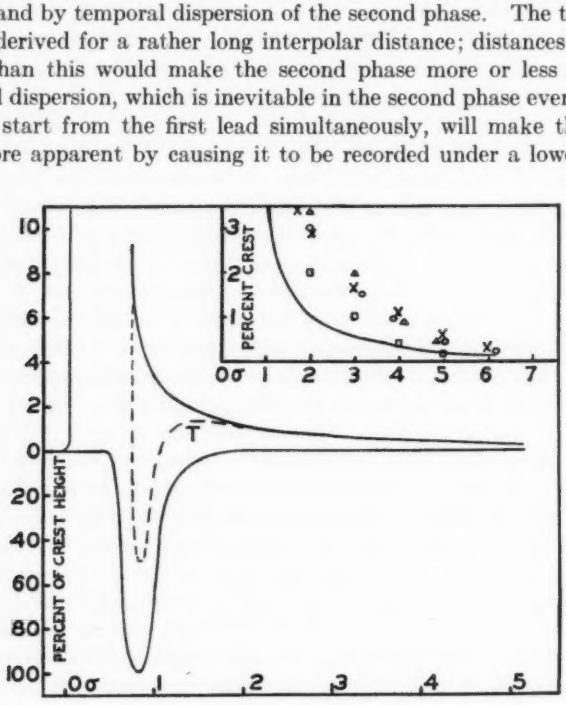

Fig. 1. Partially diphasic response reconstructed from a monophasic spike determined for the purpose, 12/5/31. Inset: Points—end of spike as measured from the level of flat veratrin after-potentials. Solid line—monophasic spike from main figure drawn in for comparison.

the potential at the first lead. In nearly all the experimental variations there is some negative potential at the end due to a residual dominance of the first phase. This results in the appearance of two crests in the axon spike potential. To distinguish the second crest from waves produced by the fiber constitution of the nerve, it has been designated the T wave for purposes of reference, in analogy with the T wave of the electrocardiogram, as the latter is interpreted on the interference theory.

Comparison of theoretical reconstructions and recorded action potentials. At constant temperature, the various experimental conditons to which a nerve may be subjected change the after-potential but leave the axon

spike form constant except for height; the potentials recorded are thus the sum of a constant[1] and a variable. In order to facilitate a clearer understanding of the various ways that the spike and after-potential may fuse, some of the possibilities are drawn in figure 2. For the portion belonging to the spike the monophasic ending shown in figure 1 is chosen as sufficiently representative; in each instance it or its diphasic derivative is added to an after-potential of arbitrarily assumed form to give the combination shown. Beside the synthetic curves is given a series of action potential records (fig. 3) made from sciatic nerves of rana pipiens under the conditions stated in the legend.

In figure 2B the after potential is assumed to be small and to have a maximum very close to the spike. With it the T wave of the spike fuses in such a way as to be undifferentiable; neither the T wave nor the end of the spike can be distinguished, the former because of the similarity of its shape to that of the after-potential at the same point, and the latter because of the close approximation of its slope to that of the after-potential. If we now imagine that the after-potential changes to the form shown in figure 2A, then the T wave emerges and the spike-end becomes visible because in the summed curve a negative slope is maintained by the spike until the latter becomes so attenuated that the slightly positive slope of the after-potential becomes dominant. The curve thus runs through a minimum (N_2). The first and second curves of figure 3 which were taken from the same nerve before (2) and after (1) treatment with veratrin have forms similar to the derived curves B and A.

In figure 2C the after-potential is assumed to rise more slowly at its start; therefore there is a suggestion of the T wave in the summation, and the slopes of the two components are just different enough to produce a change in direction of the line where the after-potential is no longer overlayed by the spike. The third, fourth and fifth curves of figure 3 resemble this type of combination.

Figure 2D may be compared with curve *6* in figure 3. The end of the spike—in this case monophasic—is not visible; nevertheless the spike may be traced to a point somewhat short of its end because of the point of inflection it causes in the curve of addition with the after-potential. It is logical to assume that the spike lasts at least until the curve begins to show negative concavity.

When the after-potential rises more slowly still (fig. 2F) the notch becomes very definite but occurs considerably before the end of the spike. This is the form of curve *7* of figure 3.

The time of termination of the spike can best be evaluated when the spike ends on a flat after-potential (fig. 2E).

[1] In practice the amount of diphasicity actually occurring in a monophasic lead may vary with the momentary condition of the nerve. This changes the recorded form of the spike and adds considerably to the difficulty of interpretation of records.

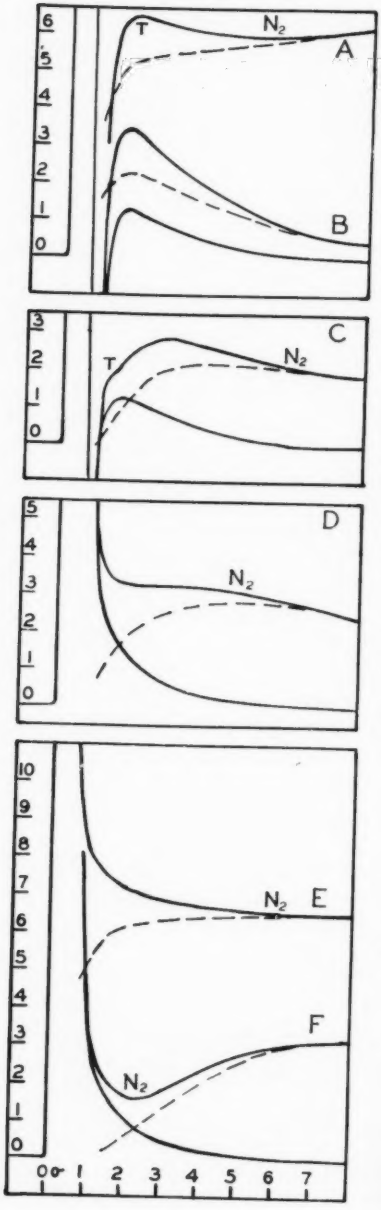

Fig. 2

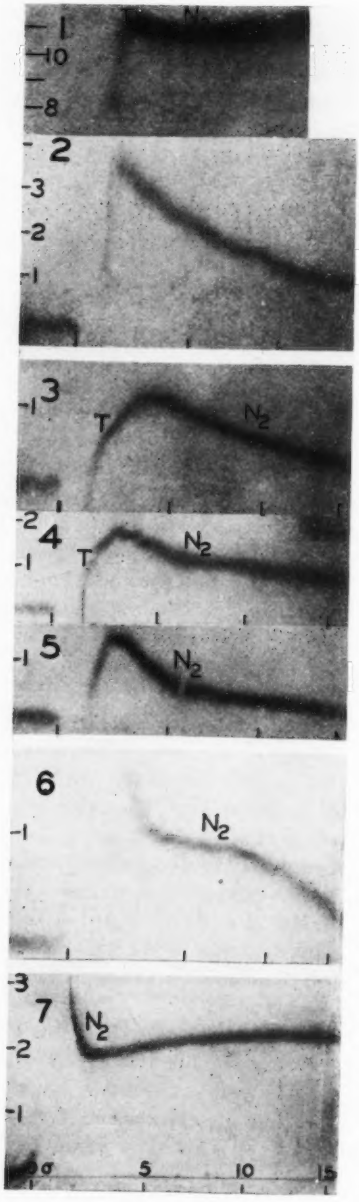

Fig. 3

Experimental examination of the origin of N_2. Using a conventional monophasic lead (nerve killed by heat at the distal electrode) a number of procedures were tried to test the hypothesis that the spike lasts at least up to the position of N_2. It was first necessary to show that N_2 is not an artifact. That it was not connected with the shock artifact was shown in a number of ways. The simplest and most direct way was to reverse the shock; this was done repeatedly in different experiments without making any significant change in the picture. The notch appeared as well when conduction was long as when it was short; and when records were made with the stimulus near the lead a comparison of them with the shock artifact recorded after subsequent narcosis, revealed that the notch could not be related to the artifact.

Another source of artifacts is the second lead, but if the notch were due to the second lead it should mirror some event at the first lead occurring earlier by the period of interpolar conduction. No event fulfilling this condition is observable; furthermore, when the nerve is so completely monophasic that no distortion at all can be detected in the spike the notch is still visible. The additional possibility that N_2 is an artifact produced by irregularities in the nerve between the leads is readily ruled out.

Theoretically, to prove that N_2 is produced by the ending of the spike it is necessary to show that it has the qualities which would be predicted from the known behavior of the spike. In going over the possible tests it was found that some of the more obvious ones were not applicable. For instance, N_2 should come later when the conducting distance is long, but its position is too ill-defined for this small difference to be measurable: The change of the form with temperature proved better, but here the si-

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Fig. 2. Action-potential curves which would be produced by combining the spike shown in figure 1 with after-potentials of various arbitrary forms. Abscissae, time in sigmas; ordinates, potential in percentage of spike height. Interrupted line, after-potential; solid line, spike alone or summed with after-potential. *A*, *B*, *C*, partially diphasic; *D*, *E*, *F*, monophasic.

Fig. 3. Action-potentials recorded under various conditions in such a way as to show the transition from the spike to the after-potential. The potential is calibrated in percentage of spike height (ordinate). The time is marked in 5 sigma intervals. Reproduction × 0.7. The spike starts at the break in the line but only its end is visible. Records *1–5*, partially diphasic; *6* and *7* monophasic. *1* and *2*, 11/23/31, 26°C., 4 mm. conduction; *2*, normal nerve, response 18 per cent of maximum; *1*, same nerve after application of veratrin and stimulation, response 11 per cent of maximum. *3*, 12/1/31, 22°C., 3 mm. conduction, normal nerve, response 60 per cent of maximum. *4*, 11/17/31, 25.5°C., normal nerve, response about 20 per cent of maximum. *5*, 12/7/31, 30°C., 10 mm. conduction, normal nerve, response 40 per cent of maximum. *6*, 1/6/32, 22.2°C., normal nerve, lead from cathode, nearly maximum. *7*, 1/7/32, 25°C., nerve treated with veratrin and stimulated. The nerve had been made monophasic with potassium and its monophasicity proven in earlier observations.

multaneous change of the spike and after-potential caused difficulty. Concomitant with the prolongation of the spike by cooling, the after-potential changed its shape so that reading the position of N_2 was difficult or impossible. When the nerve was warmed above laboratory temperature, the changes in the after-potential interfered less, and N_2 was easier to locate. On one nerve observed at 26° and 29.5°C. both the crest-time and the time to N_2 were 1.4 times as long at the lower as at the higher temperature; in another nerve, between the temperatures of 24.2° and 31°C. the coefficient was 1.5 for the spike and 1.6 for N_2. N_2 was obviously behaving as one would expect it to behave as part of the spike.

The location of the end of the spike is possible in nerves in which a change occurs in the degree of diphasicity of the lead. When the lead is monophasic a greater addition of spike potential to the after-potential occurs than when the lead is diphasic, so that as a change is made from one condition to the other the combined potential should rise and fall, pivoting about the point N_2 which is postulated to mark the end of the spike. That this is the case is shown in figure 4A. The cause of the shift from diphasicity to monophasicity in this instance was a period of tetanic stimulation. The nerve had been treated with veratrin, and on several occasions we have noted that such nerves, though recording a second phase when rested, become nearly monophasic after stimulation. The stimulation must so alter the nerve at its killed end as to cause a suppression of the response in that region and thereby remove the second phase. In the spontaneous increase in the size of the second phase that occurs in the period following a fresh inactivation of the end of a nerve by heat, the same pivoting of the end of the spike has been noted.

Another bit of evidence as to the origin of N_2 is derived from the behavior of the T wave as the after-potential changes in shape. The T wave, that is the whole area of the spike between the diphasic artifact and the end, should move as a unit as the after-potential increases. Our records contain numerous cases of such movement. None of the combinations of records which show it is reproduced, but their nature may be illustrated from the figures. The fresh nerve shows a trace of the T crest and the N_2 trough, as in figure 3, *4*; later when the after-potential is larger nearly the whole T wave becomes exposed, as in figure 3, *1*, with the crest and trough in the same positions which they held in the first record.

Duration and magnitude of the end of the spike. Two questions are of interest with respect to the end of the spike: how long does it last and what is its potential. As the spike ends asymptotically no definite value can be given to its duration,—one can only say that it lasts at least as long as it can be detected as such. The best figures for the apparent end are obtained when the spike joins an approximately horizontal potential of such size and duration that there is no doubt that it is an after-potential.

Needless to say, temporal dispersion must be eliminated either by leading from the stimulating cathode or by using small responses with only a few millimeters of conduction. A statistical check of the experiments for the time to N_2 involved the use of many in which the crest time had not been measured; therefore only those experiments were included which were performed in the temperature range from 22° to 25°C. In this temperature range the crest time is known to be about 0.3σ. The corresponding durations of the action potential up to N_2, in the great majority of the experiments, fell between $6\text{–}8\sigma$. The data justify the semi-quantitative statement that under the most favorable conditions the spike potential is evident for at least twenty to twenty-five times the duration of its rising phase.

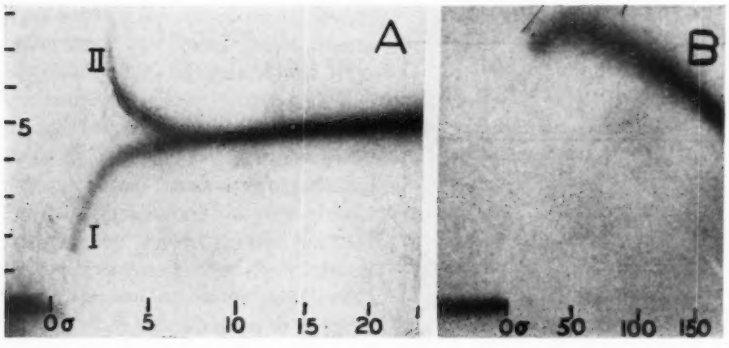

Fig. 4. A, change in form of the action potential as the lead shifts from diphasic toward monophasic. I, nerve treated with veratrin; II, same nerve immediately after stimulation. Ordinate, per cent of crest height. The pre-veratrinization form of the after-potential of this nerve is shown in figure 3, *3*. B, like A but record continued over a longer period to show the crest of the after potential. The spike height does not correspond to that in A.

The part of the spike with the steep slope, that is, up to the bend on the falling phase, which is the part measured in low amplication records, is therefore only 11 to 15 per cent of the total detectable duration.

The magnitude of the end of the spike can only be approximated as lying between two theoretical limits. The upper limit is the value of the total potential in a cool fresh nerve which contains in addition to the spike a small undetermined amount of after-potential. The lower limit is obtained in an experiment of the following type. When a nerve is treated with veratrin, the form of the spike is unchanged, in contrast to the great increase in magnitude and duration of the after-potential (9). When such a nerve is subjected to a period of rapid stimulation, the after-potential may show a nearly stationary value in the portion under the end

of the spike (fig. 5). The spike is thus lost to view on a plateau formed by the after-potential. In this combination the spike size can be estimated if the reasonable assumption be made that an extrapolation backward of the after-potential as a straight line parallel to the X axis should leave above the line a potential *at least* as great as that belonging to the spike, since from all that is known about the start of the after-potential any deviation from horizontal would be downward.

Experimentally it proved very difficult to get the right combination and attempts to do so resulted in numerous failures because the after-potentials obtained were either rising or falling. Three successful experiments are plotted as points in the inset in figure 1. The experiments were performed at 22.2 to 25°C., and in one case the crest time was measured and found to be 0.32σ. All three experiments must have had very similar crest times, and the monophasic spike calculated to 0.3σ as previously described is therefore comparable with these veratrinized nerve spike endings and is plotted as a line in the inset. Considering all the elements entering into the two methods of evaluating the potential the agreement is very good. The deviation which occurs is in the reverse direction from the one which would theoretically be expected, but it does not follow that this is a product of the methods; too few data are available for a conclusion on this point to be drawn. The action potential recorded in figure 1 has a particularly low-valued terminal portion and a comparison of it with the spike endings calculated by the other method leads to the conclusion that it can have very little after-potential in its composition. It therefore affords the closest approximation to the complete course of the monophasic spike at present available.

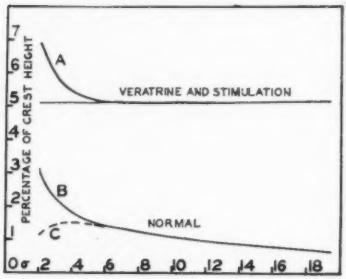

Fig. 5. A, ending of spike on flat veratrin after-potential. B, normal action potential. Both records from the same nerve, made monophasic with KCl, 1/6/32. Lead from stimulating cathode. C, residual potential after subtracting the spike value measured on A.

Because the spike form is so greatly affected by temperature its dimensions can best be designated in terms of the crest time. The early fall of the spike potential is so rapid that it drops below 5 per cent of the crest value at about 3 crest times. At this point there is a rather sharp bend in the curve and the decline becomes more gradual. This is the point which has previously been taken quite arbitrarily as an index to the spike duration. Granted that the spike of figure 1 gives the order of magnitude of the late part of the spike, 1.0 per cent of the crest potential is left at about

7 crest times and 0.5 per cent at 11 crest times. The potential becomes indeterminate at about 20 crest times.

In the determination of the spike duration temporal dispersion on conduction was controlled according to the usual methods, but the recent experiments of Erlanger and Blair indicate the possibility of dispersion from another cause. When a threshold induction shock is used for the stimulus a delay of about one crest time occurs in the setting up of the response under the stimulating cathode. The latency is less than this for stronger shocks but as long as the stimulus is submaximal there must be some fibers responding just at threshold and these might prolong the axon potential by a time which is the difference between the latencies of the most irritable fibers and of those responding at threshold. This prolongation would amount, however, only to about five per cent of the duration measured.

The start of the after-potential. By comparing the action potential endings at different temperatures it was previously shown (6) that as soon as three crest times had elapsed after the start of the action potential, the after-potential could be identified as such. This has been amply confirmed in a much simpler way in the present research; the part of the action potential which visibly changes form with alterations of the magnitude of the after-potential begins at the diphasic artifact. Comparison of records *1* and *2* in figure 3 gives a not particularly good illustration of this change.

About the form of the start of the after-potential little has been known. If the after-potential be due to catabolic products as has been proposed by Levin, the simplest inference would be that it would start at a maximum and proceed along a decremental curve as the products are removed by chemical change or diffusion. The first intimation that such is not the case was found in nerves treated with veratrin or with calcium-rich Ringer's solution. If these nerves are subjected to repeated stimulation they may show after-potentials rising in value for 50 sigmas or more (9). In general changes in tissue activity under the action of drugs are of degree rather than kind; they are exaggerations or suppressions of processes normally taking place. On this ground the expectation would be that normal nerves, too, would show after-potentials starting out at less than maximum. The evidence verifying this prediction is set forth in the following paragraphs.

One of the many forms of after-potential observed experimentally reveals by mere inspection that the after-potential does not decrease logarithmically throughout its course. This form is illustrated in figure 3, *6*. It is an axon potential obtained with a lead from a stimulating cathode on a nerve recently killed at the distal lead and having no visible diphasicity. At the start the shape of the wave is obviously due to the decremental end of the spike, but when this no longer dominates the picture the curva-

ture of the wave is downward. It is only later, in the part of the curve not included in the figure, that the curve takes on a typical decremental form. Therefore, the course of the potential is not explainable on the basis of a single chemical process. What the after-potential amounts to before the spike ends is not directly visible though it might be estimated by subtracting from the potential the mean value of the end of the spike which was derived in the first section. The result would obviously be a curve of the form assumed in the parallel reconstruction, figure 2D; but the beginning of the after-potential may be obtained with less calculation in another manner.

The early part of the after-potential is revealed in purer form with partially diphasic leads, that is, leads in which the nerve has been heated under

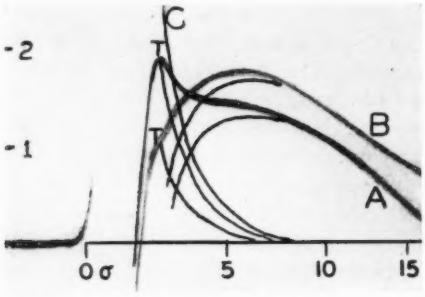

Fig. 6. Crest of the after-potential. Records of fresh nerves made with the lead at the stimulating cathode, 1/6/32, 22.2°C. Ordinates give per cent of crest height. A and B are two records, manifesting different degrees of diphasicity, printed from two films (retouched) superimposed. C is the form of the end of the spike taken from the inset in figure 1 (curve marked with circles).

the distal electrode for the purpose of obtaining a monophasic response without complete attainment of the desired result. A form frequently yielded by this type of experiment is shown in figures 3, 3 and 6B. The after-potential is recorded with a crest. Now, is this crest an artifact? The idea immediately suggests itself that the crest may be produced by the diphasicity. In this connection the T wave of the spike stands us in good stead in reaching a decision. When the T wave can be identified, we know that throughout its duration, in order to get the true after-potential picture a value must be *subtracted*, not added. The amount to be subtracted is something less than the value that the monophasic spike would have at this time; but whether one use the full value of the monophasic spike or any value less than this down to zero, the result of the correction is in the latter case to leave the form as recorded and in the former to accentuate the rising phase.

With curve B of figure 6 is included another earlier record, A, from the same nerve made when the lead was less diphasic. It identifies the T wave with unusual clearness and supports the interpretation given to the small deviation labelled T in curve B. Curve C in the same figure is a typical monophasic spike ending. The maximum amount that could be subtracted from curves A and B would be a fraction of the spike potential of such magnitude that the end of the spike would pass through the T crests. The remainders from such subtractions are drawn into the figure. Somewhere between these lines and the actual records must lie the truth of the matter. The result is a potential starting near the beginning of the nerve response and increasing to a maximum at about 7σ.

As a final illustration we may consider a purely monophasic ending from a fresh nerve in which the after-potential was small and in which the action

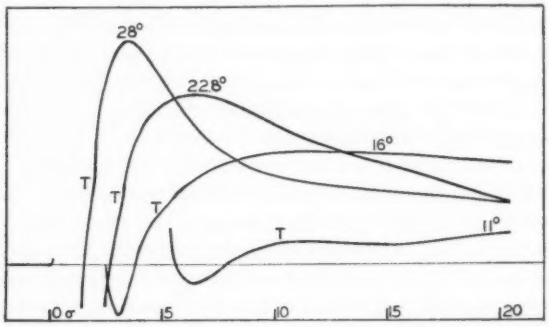

Fig. 7. After-potentials from the same nerve at various temperatures. 3/8/32. As the spike crests corresponding to these after-potentials do not have the same height the magnitudes of the after-potentials are not comparable.

potential manifested an eventless decrementation to zero (fig. 5B). When this curve is corrected for the minimum spike value as measured on the same nerve, the after-potential appears to run through a maximum under the end of the spike (5C). This is the other extreme from the condition obtaining in stimulated veratrinized nerves, and it is apparent that the crests of the after-potential may occur, according to the conditions, all the way from 3 or 4σ to 60σ or more (reference value of crest time 0.3σ).

Discussion. The observations which have been described in the preceding sections necessitate a restatement of several problems in nerve physiology.

The period of rising after-potential preceding the terminal decremental curve suggests that antecedent to the period of dissipation of the source of potential there must be a period of a different nature. Catabolites might have to diffuse over a finite distance before exerting their effect; they might

be formed over a sufficient time interval to show augmentation of potential through accumulation; or they might have to be metabolised to some potential-producing intermediate, an oxidation product, perhaps, since as first shown by Amberson the after-potential is greatly depressed in asphyxiated nerves. It may be however that oxygen is necessary not for such an oxidation of catabolites, but rather for the maintenance of a state of the nerve in which the catabolites can produce a potential.

The explanation of the interval of rising after-potential on the basis of a diffusion time is insufficient, as the amount of change is too great to be accounted for on the basis of the temperature coefficient of the rate of diffusion in aqueous systems. (Because of the tendency of the after-potential to change independently of temperature, no attempt has been made to determine the coefficient. Its order of magnitude is often found to be as large as or larger than the one holding for the spike.)

The second and third suggestions concerning the period of rising after-potential postulate underlying chemical processes and therefore demand a positive temperature coefficient; but chemical hypotheses have met a difficulty in the fact that the temperature coefficient of the after-potential duration is negative. The latter however gives an incomplete picture of the behavior of the after-potential as a whole, for the development of the potential occurs more slowly when the nerve is cooled (fig. 7). Thus, as far as the genesis of the after-potential is concerned, the modification by temperature is compatible with a chemical hypothesis, for the necessary prolongation of the time of the production of secondary catabolites in cooled nerve is found. The shorter total duration however would have to be explained on another basis. A slower rate of production of metabolites might lead to a smaller accumulation, as their removal by diffusion would have a lower temperature coefficient and would therefore be relatively more effective at lower temperatures. Furthermore the surface film may be more stable. Fewer molecules would move into and out of the surface in unit time and the surface would be less susceptible to modification by addition of foreign molecules. Thus the potential would be less altered by a given concentration of a molecular species, and the concentration might fall below the threshold of potential production earlier in spite of a slower restoration to the normal resting chemical composition.

A problem whose solution is more remote is the nature of the origin of the two potentials. In addition to the previously known factors in this problem we now have the condition that the potentials occur simultaneously and without any parallelism, one may be rising while the other is falling. There is to be explained not the nerve potential but the nerve potentials. Are we to assume two chemical processes, one causing a high short-lived potential and the other a low prolonged one, both through the action of metabolites altering the constitution of the surface film or setting up

liquid chain potentials as assumed by Levin for the after-potential; or are we, in view of the striking differences in the qualities of the two potentials, to regard the spike as due to a transient interruption of the surface film according to the inherent supposition of the classical theory and to grant that the after-potential only has a chemical basis? Both views can be defended and a decision between them is impossible.

The tail on the spike calls for a reconsideration of the relation of the relatively refractory period to the spike. The fact that the nerve is absolutely refractory until the spike potential has almost subsided (Adrian), taken with the apparent absence of potential during the relatively refractory period, leads to the conclusion that there is no relation between the refractory period and the spike. Now we find that the spike potential is easily long enough to last throughout the relatively refractory period. Even so, there is still no very great parallelism between the course of return of irritability and the restoration of the normal potential, but the discrepancy finds a possible explanation in the recent observation by Bishop (1931) that the nerve fails to conduct when the resting potential is depressed 2 to 3 millivolts by various means. Irritability would not be expected to return at all until the depression of the resting potential by the action wave is less than this amount (absolutely refractory period), and the rise of irritability to normal would be simultaneous with the restoration of this final small amount of potential (relatively refractory period). Quantitative studies in this direction are needed.

Gasser and Erlanger observed that in cold frog nerve the supernormal phase disappeared and the relatively refractory period lasted as long as the action potential. On the basis of this observation they made the suggestion that in this state all the potential might belong to the spike. This surmise with respect to the nature of the potential has been confirmed in the present series of experiments by observing the course of the two potentials over a series of temperatures, as the after-potential disappeared and the spike approached its pure form in the limit. Taken together the two observations support the possibility of correlation between the spike and refractory period.

No complete correlation is possible, however, as the refractory period is known to be modified under various abnormal conditions which are not known to change the spike form. Part of the variation may be associated with the after-potential. In its later course the latter is associated with the supernormal phase; and, since it can be demonstrated to exist as early as the portion of the spike at which the absolutely refractory period ends, it might be expected to alter nerve irritability during continuance of the spike. Another factor which may alter the relatively refractory period is one which occurs without any potential sign. Following a subthreshold cathode shock, and therefore in the absence of a spike, Erlanger and Blair

have described a period of depression having the same duration and the same behavior with respect to temperature as the relatively refractory phase.

SUMMARY

The form of the axon spike potential has been followed to a point closer to its end than in previous determinations.

The potential of the falling phase drops below 5 per cent of that obtaining at the crest in about 3 crest times. In this region the rate of decline shows a sharp decrease so that the phase ends along a slowly decrementing curve and becomes indistinguishable at 20 to 25 crest times.

When the potassium method is used for making the nerve monophasic the potential recorded is found to be much freer of diphasicity than when the indifferent lead is produced by heat coagulation. The form of the monophasic spike obtained with the potassium method is shown.

New evidence is given that in the ordinary "monophasic" lead the first phase outlasts the apparent position of the diphasic artifact. The wave which this causes in the records is labelled *T*.

During at least 85 to 90 per cent of the visible duration of the spike potential the nerve shows signs of the production of another potential very different in its general behavior, the after-potential.

The after-potential has a rising phase. Like the total duration this rising phase lasts for very variable times, according to the condition of the nerve. Its crest may occur at 3.0 to 4.0σ (estimated) at the lower limit up to 60σ or more at the upper (temperature range 20–25°C., comparative value of spike crest-time 0.3σ).

Cooling causes a prolongation of the rising phase of the after-potential.

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HISTAMINE AS THE HORMONE FOR GASTRIC SECRETION

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Since Edkins (1906) proposed the "gastric" theory of gastric secretion, there has accumulated considerable evidence indicating the existence of such a hormone mechanism. The existence of a humoral mechanism has been definitely established by the transplantation experiments of Ivy and Farrell (1925), Lim, Loo and Liu (1927), and by vivi-dialysis experiments of Necheles and Lim (1928). Koch, Luckhardt and Keeton (1920) have attacked the problem of the isolation of the active principle of extracts of pyloric mucosa. Although they did not succeed in isolating the active substance, they did discover a number of its chemical properties and stated that histamine and gastrin "may later be found to be identical or closely related."

We (Sacks, Ivy, Burgess and Vandolah, 1931) have been able to isolate histamine as a sulphate from the pyloric mucosa of the hog under conditions which preclude the possibility that it is present as a result of putrefactive changes. We have obtained other evidence showing that histamine is the only gastric secretory excitant present in extracts of the pyloric mucosa which is active subcutaneously. Our evidence indicates either that histamine is the gastric hormone, or if not, there is no gastric hormone, or the gastric hormone has never been extracted from pyloric mucosa.

Isolation of histamine. The washed pyloric mucosa of the hog, obtained as it came down the chute at the slaughter house within thirty minutes after the animal was killed, was immediately subjected to extraction with 1 per cent sulphuric acid in 80 per cent alcohol. The extraction was allowed to continue overnight, the acid liquid siphoned off, and replaced by a fresh portion. In all, three extractions were made. To the alcoholic solutions, sufficient activated charcoal was added to remove pigment and suspended material. It was found that under these conditions the activated charcoal (Mallinckrodt) did not adsorb any of the active secretory or vaso-depressor substance. After separating the charcoal by filtration, Lloyd's reagent (hydrous aluminum silicate, Lilly) was added to the clear filtrate in the proportion of 1 gram per 100 cc. The mixture was shaken

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thoroughly and the Lloyd's reagent then allowed to settle. The supernatant fluid was siphoned off and discarded. The Lloyd's reagent was then sucked dry at the pump and washed with aqueous 1 per cent sulphuric acid. It was then extracted at room temperature with 3 per cent aqueous ammonia. The ammoniacal extract was filtered by suction, evaporated to a small volume, made slightly alkaline with sodium hydroxide solution and mixed with 1½ times its weight of anhydrous sodium carbonate. On standing overnight this became a hard cake. This cake was powdered in a mortar and extracted with chloroform in a continuous extraction device. The extraction was continued until fresh extracts no longer gave a Pauly reaction.

The combined chloroform extracts were filtered to remove some dark gummy material and then extracted with a small amount of water. The aqueous extract was filtered and boiled to remove chloroform and volatile bases. The solution was then made just acid with nitric acid and silver nitrate in slight excess was added. The precipitate which formed was separated by centrifugalization, and discarded. The mother liquor was then made neutral with barium hydroxide solution, when a small amount of dark brown precipitate formed. This too was removed by centrifugalization and discarded. To the remaining solution barium hydroxide was added in excess. This precipitated the histamine as a silver salt. The precipitate was washed free of barium suspended in water, and dilute HCl added in slight excess. This converted the histamine-silver into the soluble histamine hydrochloride and insoluble silver chloride. The latter was removed by filtration and the filtrate evaporated to a small volume. On standing overnight, a small amount of crystalline material separated from the solution. This was dissolved in 95 per cent alcohol (twenty volumes) and concentrated sulphuric acid added drop by drop, to maximum precipitation. The needles of histamine sulphate were separated by filtration and washed with alcohol and ether.

The histamine was identified by conversion into the dipicrate. The rhombic yellow leaflets obtained melted at 230–232°C. When mixed with dipicrate from known histamine, the melting point was not depressed. This method has been repeatedly applied successfully to different original extracts.

During the early part of our work we repeated the chemical studies of Koch, Luckardt and Keeton (1920) and confirmed them in every particular. They found that chloroform failed to extract "gastrin" from an aqueous alkaline solution. This is true when chloroform is shaken with an alkaline solution of "gastrin." However, chloroform will extract the active principle when the latter is mixed with anhydrous sodium carbonate and the dry, powdered mixture subjected to continuous extraction with chloroform. Although the yield is only about 20 per cent, chloroform is

superior to amyl alcohol in that the chloroform extracts less impurity. The amount of activity destroyed varies. We also found that "semi-pure gastrin solutions" failed to yield an active picrate or picrolonate as does histamine. But more purified solutions sometimes yield a picrate. For example, on one occasion 1200 doses of "gastrin" obtained by chloroform extraction were precipitated with silver nitrate and barium hydroxide, the silver being removed by hydrogen sulphide and the barium by sulphuric acid. The solution assayed 500 doses of "gastrin." The solution was then concentrated to a small volume and saturated sodium picrate solution added in excess. On standing, a yellow crystalline precipitate was obtained which was filtered off on a Hirsch funnel and recrystallized twice from water. One hundred and fifty-five milligrams of crystals were obtained and 1 mgm. gave a good gastric response. The melting point was 217–231°; mixed melting point with known histamine dipicrate was 217–225°. No crystals were obtained from hot alcohol, but on evaporation of the alcohol and taking up the residue in hot water, crystals again formed having a melting point of 221–224°. On blood pressure assay the crystals reacted as histamine picrate. It should be added that on several occasions we were able to obtain histamine crystals from intestinal mucosa by the methods of Barger and Dale (1911) and Abel and Kubota (1919), but were unable to obtain histamine crystals by applying these methods to pyloric mucosa.

Histamine, the sole gastric stimulant present in the extract? Since the yield of crystalline histamine is rather small, it became necessary to ascertain if histamine is the only substance present in the original extract which stimulates gastric secretion, when administered subcutaneously. This was done in two ways: first, by comparing the intensity of the Pauly reaction, the vaso-depressor action, and the gastric secretory stimulation of the solutions at each step of the extraction process, with the same properties of pure histamine, and second, by using histaminase (1930). The original alcoholic extract gives a more intense Pauly reaction than would be expected from the physiologic actions, but the secretory and vaso-depressor activities were in the same ratio as in all subsequent steps. (Vandolah has found that thoracic duct lymph after feeding gives a good Pauly, but does not depress blood pressure or stimulate gastric secretion.) The Pauly reaction is not an accurate index of vaso-depressor or "gastrin" activity under all conditions. We use the term "ratio" because when "impure gastrin" and histamine having equal secretory effects are given intravenously, the depressor effect of histamine is greater. The solution left after the histamine is adsorbed on the Lloyd's reagent still gives a Pauly reaction, but has no vaso-depressor or gastric secretory effects. However, solutions at the subsequent stages of the process gave vaso-depressor and gastric secretory responses which ran parallel with each

other and with the intensity of the Pauly reaction. This must be interpreted as good evidence that histamine is the sole excitant of gastric secretion present in the extracts (active subcutaneously). The other possibility, that there is another substance present with the same ratio of vaso-depressor and secretory activities as histamine, is rather remote.

Histaminase destroys "gastrin." The second method of showing that histamine or a very closely allied imidazole is the only excitant of gastric secretion present in the original extract, made use of the histamine-inactivating enzyme of Best and McHenry (1930). A quantity of the original acid alcohol extract of the mucosa was evaporated to remove the alcohol, taken up in water, brought to pH 7 with sodium hydroxide, and incubated with histaminase powder made from the kidney under toluene at 37° for 24 hours. The quantity of histaminase used was twice that necessary for the amount of histamine shown to be present by blood pressure assay. After the incubation, the solution was filtered, evaporated to remove toluene, and the entire quantity injected subcutaneously into a Pavlov pouch dog. Although, on the basis of previous gastric secretory assay, the solution had originally contained ten times the effective dose of histamine, there was no response by the pouch. The usual dose of histamine (0.5 mgm. of the phosphate) injected into the same dog two hours later, gave the customary response. Thus we can say from this experiment (which was repeated ten times with the same results) that the active secretory agent in extracts of the pyloric mucosa can be inactivated by histaminase, which shows that the active principle is histamine or a very closely related imidazole.

Specificity of histamine. A variety of other imidazoles have been tested for gastric secretory activity, with negative results except in the case of pilocarpine and isopilocarpine (Sacks, unpublished). Koch, Keeton and Luckhardt (1920) used histidine, methylimidazole and hydroxymethylimidazole; Burgess and Ivy (1930) used imidazole, imidazolealdehyde, imidazole proprionic acid, and imidazole lactic acid in 5 mgm. doses; all yielded negative results. Methylene histamine made by the action of formaldehyde on histamine, kindly furnished by Doctors Kendall and Gebauer, was also found to be ineffective in doses of 5 to 10 mgm. Pilocarpine and isopilocarpine stimulate salivary and pancreatic secretion and comparatively large doses are required to stimulate gastric secretion. Hence histamine may be said to be the only known imidazole which is a "specific" gastric secretory stimulant.

Is a gastric secretory excitant present in urine? As imidazoles are known to be present in urine, we investigated to ascertain whether a gastric secretory excitant could be obtained from that source. We collected the urine of men that was formed following the ingestion of a meal and subjected it to treatment with Lloyd's reagent. In one experiment out of three, the

material obtained from one liter of urine produced a slight augmentation of gastric secretion (table 1); thus a threshold dose was present. This agrees with the work of Best and McHenry (1930) who report on the basis of vaso-depressor assay, 0.2 mgm. of histamine per kilogram of urine.

On the vaso-depressor effect of the hydrous aluminum silicate-histamine compound. Since it is possible that histamine may be bound with some inert substance that would prevent it from being precipitated with picric, picrolonic and flavianic acid in certain solutions, but still permit it to exercise its vaso-depressor and secretory effects, we decided to treat some histamine with Lloyd's reagent and determine its vaso-depressor effect. It was found that histamine when absorbed to Lloyd's reagent still possessed its vaso-depressor and secretory properties, but to a somewhat less degree.

TABLE 1

Showing the presence of a gastric secretory excitant in human urine post-cebum active on subcutaneous injection

Urine extract 30.0 cc. equivalent to 1 liter of human urine.

PROCEDURE	TIME	VOLUME	FREE HCl	TOTAL HCl	OUTPUT HCl
	minutes	*cc.*	*per cent*	*per cent*	*mgm.*
Control	60	2.5	0.1824	0.3554	8.885
Urine extract 30.0 cc., subcutaneous injection					
Post-injection	60	4.0	0.3007	0.3646	14.584
	60	3.5	0.1459	0.2280	7.980

Discussion. The literature on the question of the existence of a hormone mechanism for gastric secretion has been reviewed by one of us (Ivy, 1930, 1931); hence, it will not be referred to in detail here. It is sufficient to state that although it has been established that a humoral mechanism is concerned in gastric secretion, the nature of the humoral agent or agents has not been determined. The humoral agent may be either a hormone, or secretagogues, absorbed from the lumen of the bowel, or both.

The active principle of dilute acid extracts of the pyloric mucosa has been regarded as the gastric hormone "gastrin," since its discovery by Edkins (1906). However, a gastric secretory excitant has been found in extracts of many animal tissues and plants, especially in such tissues as duodenal mucosa, liver, pancreas, and thyroid (Koch et al., 1920; Ivy, 1930). The fact that all such tissue extracts on intravenous injection caused a decided fall in blood pressure led Popielski (1920) to refer to "all the secretory excitants" extractable from tissues as vaso-dilatins and to regard them as non-specific. The widespread occurrence of histamine

explains the widespread distribution of "gastrin bodies" or "gastric secretins."

However, the rather ubiquitous distribution of histamine does not necessarily discredit the probability that histamine is the gastric hormone, since it is well known that the admixture of a little vaso-dilating agent to vaso-depressor free secretin augments the pancreatic secretory response, that local vaso-dilatation is favorable for secretion in general, and that histamine may be injected intravenously at such a rate as to obtain gastric secretory stimulation without a general vaso-depression (Ivy and Javois, 1924; confirmed by Ivy and Kim, unpublished). As a matter of fact, histamine if produced in physiological amounts by the pyloric mucosa during digestion, may be viewed as not only being a specific hormone augmenting and facilitating the formation of gastric juice, but also as being a "general digestive hormone" augmenting the formation of pancreatic juice, bile, and succus entericus (Koskowski and Ivy, 1925) and increasing the motor activity of the gastro-intestinal tract.

This view is supported by the observation of Best and McHenry (1930) on the distribution of histamine and histaminase in the tissues of the body. They find that the stomach and liver are the only organs (the thyroid has not been examined for histaminase) examined in the body which contain relatively large quantities of histamine which do not also contain histaminase. The enzyme is absent from the stomach and practically absent from the liver. From their work we can say that it is easily possible for the pyloric mucosa to liberate enough histamine into the portal blood and then into the general circulation to stimulate the production of acid by the gastric glands and to exercise a generally synergistic action on the digestive process.

The crucial question that arises in regard to the preceding discussion is: "Does histamine get into the blood during digestion?" Necheles and Lim (1928) obtained from the portal blood by the method of vivi-dialysis some agent that unquestionably stimulated gastric secretion on subcutaneous or intraperitoneal injection. They determined the blood pressure effect when they tested the dialysates for the presence of a pancreatic excitant and found that they stimulated the pancreas, causing but little depressor effect. The reports of these investigators are not entirely clear on the vaso-depressor activity of their portal dialysates. We have also made dialysates and have extracted "fed" and "unfed" blood. We find a vaso-depressor agent that is not an inorganic salt. But the results of our chemical and biological studies are as yet not sufficiently clear cut to warrant a definite statement. Hence the question asked above, we believe, cannot be answered with the evidence at hand. However it should be pointed out that Best and McHenry report that a small quantity of histamine is present in the blood (0.4 mgm. per kgm.) at all times. Further, the observations

of Koskowski and Kubikowski (1929) indicate that the histamine content of the blood is increased during the gastric digestion of meat.

Two other questions that must be considered are: Is histamine the sole active agent of acid extracts of the pyloric mucosa? And, might not the chemical procedures used have produced histamine from a closely allied imidazole? Koch, Luckhardt and Keeton, as a result of their chemical studies stated that histamine and "gastrin" may later be found to be identical or closely related. We adhered to this belief until we had actually obtained histamine crystals from a number of extracts and had checked the vaso-depressor and secretory effects of all fractions. One reason why we believed them not to be identical was that histamine in proper concentration is precipitated uniformly by picric and picrolonic acid, but "gastrin" is not precipitated from certain "gastrin" solutions by these precipitants. If histamine is added to a "gastrin" solution, picrolonic acid precipitates the added histamine (Koch, Luckhardt and Keeton, 1920). But picric (Koch, Luckhardt and Keeton) and flavianic acids precipitate the added histamine but poorly. We confirm Koch, Luckhardt and Keeton in regard to the action of these precipitants. Some interfering substance is evidently present which increases the solubility of histamine picrate and prevents it from being precipitated without interfering with its biological action. We believe that the biological evidence showing that the ratio of vaso-depressor to secretory activity is practically constant in all fractions from the original extract to the final step yielding crystals and mother liquor, is strong, if not conclusive, evidence indicating that histamine is the sole active secretory agent of acid pyloric extracts and that histamine was not produced by the chemical procedures employed.

Although histaminase abolishes the secretory and vaso-depressor effect of acid extracts of the pyloric mucosa, this does not prove that the active principle is histamine, since it has not been shown that histaminase acts on histamine exclusively. However, since histamine is the most potent vaso-depressant and gastric secretory excitant of all known imidazoles and of all other known substances, the inactivation of "gastrin" by histaminase constitutes strong presumptive evidence that histamine and "gastrin" are identical.

The evidence at hand, which appears to us to be complete, shows that histamine is the sole gastric secretory excitant in dilute acid extracts of the pyloric mucosa and that histamine may be the gastric hormone. *It has not been proved that histamine is the gastric hormone; neither has it been proved that there is a gastric hormone.* The final answer to these questions awaits the chemical isolation and identification of the active substances in the blood or in dialysates and the extraction of the principle or principles from the mucosa of the gastro-intestinal tract.

SUMMARY AND CONCLUSIONS

1. Histamine has been isolated from acid extracts of the pyloric mucosa as a sulphate and picrate. The method is described.

2. Vaso-depressor and secretory assays on all fractions from the original acid extract to the final product of histamine crystals and mother liquor yield strong, if not conclusive, evidence that histamine is the sole secretory excitant active subcutaneously in acid extracts of the pyloric mucosa and that histamine was not produced by the chemical procedures employed.

3. Histamine (0.5–1.0 mgm.), pilocarpine (5–10 mgm.) and iso-pilocarpine (10 mgm.) are the only imidazoles out of ten tried that stimulate gastric secretion. Methylene histamine (5–10 mgm.) does not stimulate gastric secretion.

4. A threshold dose of a gastric secretory excitant is present in 1 or more liters of urine which is to be expected on the basis of the histamine content of urine (Best and McHenry, 1930).

5. Histaminase destroys the gastric secretory effectiveness of "gastrin" solutions.

6. Histamine adsorbed to Lloyd's reagent maintains its vaso-depressor and secretory properties.

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FATIGUE IN SKELETAL MUSCLE IN RELATION TO THE FREQUENCY OF STIMULATION[1]

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The following experiments were begun in the fall of 1928 as a control study for an investigation as to the nature of the muscular asthenia associated with the complete removal of the adrenal glands. In view of the complexities disclosed by the control experiments the latter problem has been indefinitely postponed, but the behavior of circulated skeletal muscle of the cat in response to stimulation at varying frequencies exhibits points of independent and timely interest. In particular it seems to afford an explanation for certain phenomena recently described by Briscoe (1931) in the course of her search for a mode of stimulation which would reproduce normal postural activity.

APPARATUS AND METHODS. Most of the experiments were performed upon the circulated gastrocnemius or soleus muscles of the cat, and in most cases ether anesthesia was employed, until we became aware of the differences which might be introduced by too deep an anesthesia. Subsequent experiments performed with decerebrate preparations yielded results indistinguishable from those obtained from animals under light ether anesthesia. Muscular contractions were recorded isometrically, or nearly so, by means of a torsion myograph of the type described by Fulton (1926). The torsion member is a piece of tool steel 75 mm. long and 6 by 1.5 mm. cross section. The recording arm of the myograph projects into the eyepiece of a Cambridge string galvanometer. The latter is connected to the muscle through suitable electrodes, so that we obtain a simultaneous record of the mechanical and the electrical events in the muscle. The magnification of the shortening of the muscle was approximately 40 × in experiments on soleus, and 150 × in the case of the gastrocnemius. The recording arm was provided with two vertical fibers, so spaced that when the shadow of the first was about to leave the edge of the photographic record the second appeared on the opposite edge, thereby doubling the effective width of the recording surface.

Fixation of the leg of the cat was obtained by two drills, one passing

[1] A preliminary report of these experiments appeared in This Journal, xciii, June 1930.

through the shaft of the tibia at the junction of the lower and middle thirds, and the other through the lower end of the femur for fixation of the gastrocnemius or through the head of the tibia for fixation of the soleus. These drills were rigidly clamped to a heavy animal table of adjustable height.

The sciatic nerve was severed in the popliteal space, and the branch serving the soleus and gastrocnemius muscles identified. When the activity of the soleus muscle alone was to be recorded, the nerve was usually dissected free where it passes through the gastrocnemius muscles, and all branches to the latter severed. The tendons of the two muscles were always dissected free from one another and also from the other muscles contributing to the Achilles tendon. This provided essentially a circulated neuro-muscular preparation, free from reflex activity, having fairly stable blood pressure and remaining in good condition for many hours. In the more recent experiments the blood pressure was recorded during the myographic observations. Evaporation from the surface of the muscle was minimized by smearing it freely with petrolatum or wrapping it loosely with a layer of moist and then a layer of dry cotton. Its temperature remained at approximately 30°C., or a little higher, throughout the experiment. Action currents were led off to the string galvanometer through agar electrodes ending in cotton wicks, one of which was stitched lightly to the surface of the lower third of the muscle and the other to its tendon. This yielded simple diphasic action currents. In later experiments fine silver needle electrodes were substituted for the agar electrodes.

For stimulation, shielded electrodes of the Sherringtonian type, or, in later experiments, tubular electrodes (cf. Forbes, Davis and Lambert, 1930) were applied to the motor nerve. An induction coil of the type described by Bishop (1927), with an iron core, furnished shocks strong enough to be definitely supramaximal on both make and break. These were delivered to the nerve at frequencies controlled by the rotary interrupter described by Forbes (1921). In the early experiments the frequency was varied by changing the speed of the interrupter, but in later experiments an instantaneous doubling or halving of the frequency was obtained by switching the connections to a different pair of brushes while the interrupter continued to run at a steady speed. When intermittent tetanic stimulation was required, the stimuli were periodically short-circuited by means of a mercury switch operated by a metronome. Still more recently a neon tube or helium tube stimulator closely similar to the one described by Briscoe and Leyshon (1929) has been employed.

Preliminary observations. At the beginning of these experiments we were surprised and somewhat disconcerted to find ourselves unable to demonstrate a clean-cut angle at the termination of the single muscular twitch as described by Fulton (1926). We devoted considerable time to

improving the fixation of our preparations and their physiological condition in efforts to obtain the angle, the plateau associated with it, and also a smoothly fused tetanus at low frequencies of stimulation. From time to time we obtained humps in the early phases of relaxation, but the most pronounced angle which we ever obtained was described in our protocols at that time as being only "fair." We did not discover that the angle was due to friction in the bearing of the myograph lever, as Cooper and Eccles have demonstrated (1930), but our own experience leads us to concur absolutely in this interpretation of the phenomenon in the case of the single twitch in fresh muscle. We now use a myograph whose bearing is a knife edge rocking in a shallow rounded depression instead of the original cylindrical rod resting in a V notch. Even with this myograph, however, the end of a tetanus is often marked by a relatively sudden change in the rate of fall of tension. At the end of two or three minutes of stimulation at relatively low frequency this discontinuity is most marked and forms a definite "angle" in Fulton's original sense. Under these circumstances it cannot be due to friction in the myograph, since it is obtained with a frictionless lever which shows no angle on a single twitch. Furthermore the tension is definitely falling previous to the angle, the curve being slightly concave downwards or practically linear. This slow fall then merges more or less abruptly into the familiar logarithmic curve which is concave upwards. In the case of single twitches or very brief tetani the first phase of slow relaxation is poorly developed and nothing appears but a point of inflection in the curve of relaxation.

In concordance with the work of Cooper and Eccles, we found that the tension developed in a brief tetanus in both soleus and gastrocnemius is greater the greater the frequency of stimulation up to approximately 40 or 50 per second for the soleus and to somewhere above 100 per second for the gastrocnemius. Frequencies of stimulation of this order of magnitude are required to obtain a smooth tetanus with a fresh muscle. In all respects in which we have performed similar experiments, our results agree qualitatively and quantitatively with the descriptions and values given by these workers, except that in our experience the time to the maximum of tension of a single twitch is even more variable than their data indicate. We did not pursue these aspects of the question in great detail, however, as our interest centered in the behavior of the muscles during sustained contractions, and it soon became evident that a muscle which had been in activity for a minute or more would usually give a smoothly fused contraction at a rate of stimulation far lower than that which was required at the beginning of the experiment.

The formation of a smooth tetanus at low frequencies depends upon the development of a slow initial phase of relaxation as described above. In the case of the gastrocnemius the interval between the last electrical re-

sponse and the beginning of rapid relaxation is about 65 σ at the end of a minute of stimulation. This means that a frequency of 16 per second may give a tetanus which is not grossly unsteady. The corresponding figures for the soleus are 100 to 120 σ and 9 or 10 per second. Most of our experiments have been performed with frequencies of approximately 50 and 25 per second for the gastrocnemius and approximately 30 and 15 with the soleus. The lower frequency in each case will usually give a smooth or very nearly smooth tetanus in a muscle which has been in activity for a minute or so, while with the higher rate we obtained a contraction from the fresh muscle nearly as powerful as those elicited with much higher rates of stimulation.

Preliminary experiments showed that the exact length at which the muscle was called upon to work had little effect upon the behavior or condition of the muscle, provided it was not stretched beyond the initial length to which it may be extended by flexing the ankle to the maximum degree possible in the intact animal. Between this limit as one extreme and the point at which the muscle is extended only one or two millimeters beyond the point just sufficient to take up all slack in the tendon, the total tension developed *in a tetanus* is nearly independent of the initial length of the muscle. There is a slight increase with increasing initial length, but in one experiment with the soleus this was less than a 20 per cent increase on going from the point of just perceptible initial tension to well beyond the physiological limit. In the case of the gastrocnemius the increased tension due to increasing the initial length is somewhat greater than in the soleus. If either muscle has once been extended beyond the physiological limit, irreversible or slowly reversible changes take place which make it impossible to reproduce quantitatively results obtained previously with a lesser degree of extension. We therefore performed most of our experiments at an initial extension sufficient to stretch the muscle about a millimeter beyond the point of slackness. This corresponds roughly to the condition at semi-flexion of the ankle joint in the intact animal.

SUSTAINED ACTIVITY IN RELATION TO FREQUENCY OF STIMULATION. If the circulated soleus muscle be stimulated (indirectly) at a rate of 30 per second, the tension will continue to increase for several seconds at the beginning of stimulation. This tension is then maintained for a short time but at the end of a minute the tension has fallen perceptibly. It continues to fall, at first more rapidly and then more slowly, for the next two minutes or more, finally reaching a level of perhaps 1100 grams as compared with an original maximum of 2600 grams. The absolute values, of course, vary with the size and condition of the animal. This low level of tension will then be maintained without further decrease, if the blood pressure is good, for half an hour or more. The tension associated with this approximately steady state we shall speak of as the "fatigue level."

If, instead of a rate of 30 per second, we employ stimuli at 15 per second, the tension rises less rapidly and does not reach such a high maximum. At first the contraction is not perfectly fused but shows clearly the individual humps corresponding to the separate impulses. As stimulation is continued these humps smooth out and gradually become completely or almost completely fused. The tension eventually falls somewhat, but to a lesser degree than with the more rapid rate of stimulation. Thus with the slower stimulation the tension is at first lower and later higher than it is with the more rapid rate. This relation of tension to frequency of stimulation is expressed graphically in figure 1, in which are plotted the maximal tensions and also the fatigue levels attained by a single muscle at various frequencies of stimulation. This is the most complete and best controlled of several experiments of this type. The blood pressure rose gradually from 87 to 104 during the first seven observations and remained within ± 2 mm. of this value for the remainder of the experiment except for a sudden transient fall to 84 during the observation at the frequency of 61 per second. The muscle was stimulated for periods of three minutes and allowed to rest for five. The frequency was systematically increased by steps from 9 per second to 40 per second, then decreased in reverse order, then alternated between 24 per second and the high frequencies of 52 and 61 per second. The results show lower fatigue levels at frequencies above 40 per second. This figure is unusually high for the point above which tension becomes less, but this may be due in part to the excellent general condition and circulation of this particular preparation. Another type of curve sometimes met with is shown in figure 2. The relationship of fatigue level to frequency of stimulation shows a clear maximum at 15 or 20 per second and a second less clearly defined maximum at about 40 per second. The genesis of these maxima and also the reason for the divergence of the curves in figure 1 at low frequencies of stimulation will be considered below.

An attempt was made to establish a similar curve for the gastrocnemius, but it became evident that there were so many possible variables in the situation that it would be difficult to obtain a "typical" curve and that any absolute values would be of little significance. However, by piecing together observations from a number of experiments we may say with confidence that qualitatively the same relationships hold as for the soleus and that the "speed" of the muscle is about double that of soleus, (i.e., the fatigue sets in in half the time, the optimal rate of stimulation and the rate necessary for fusion, are both about double, etc.). The gastrocnemius does not lend itself as readily as the soleus to this type of experiment. It is more difficult to attain an even approximately "steady" state or fatigue "level," for the tension usually continues to fall slowly but steadily. Furthermore, after two minutes of steady stimulation, followed by five

minutes of rest, recovery is far from complete, so that it is impossible to make many observations on the same muscle under comparable conditions. In view of the greater expenditure of energy in this muscle this situation is not surprising, and it is significant that the muscle specialized for phasic activity often fails to maintain a steady state during continuous activity while the postural muscle (soleus) rarely fails.

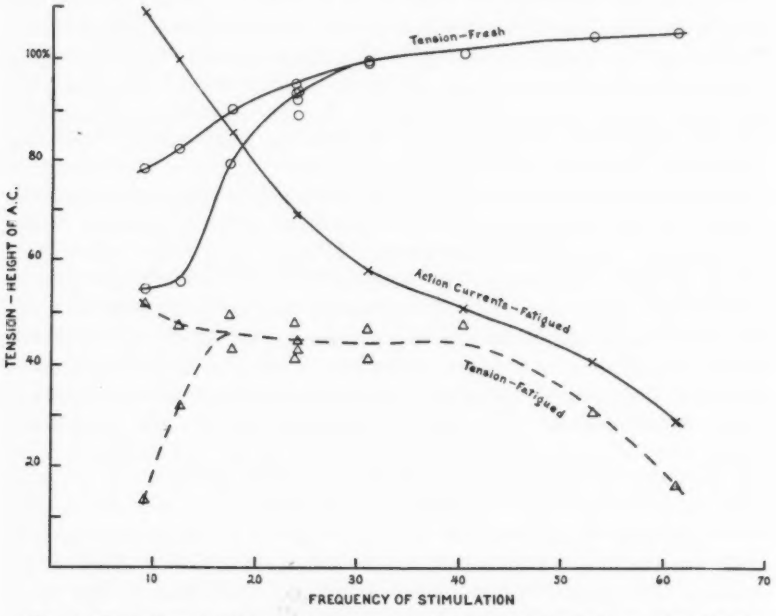

Fig. 1. December 4, 1931. Soleus muscle. Stimulation by helium tube stimulator. Frequency of stimulation expressed in shocks per second. Isometric tension developed by muscle expressed as percentage of the tension developed in a test stimulation at frequency of 31 per second 1 minute previously. Height of action currents expressed as percentage of the height of the first action current of the series in question. Observations made in regular sequence beginning with slowest frequency, going to highest and returning to low frequencies again. The well sustained tensions at very low frequencies represent the performances at the beginning of the series. Other details in text.

Among the variables which render performance of fatigued muscle difficult of precise definition, whether in the gastrocnemius or the soleus, are changes in the time values characterizing the individual muscular twitch. The time to maximum of tension has generally been implicitly accepted as relatively constant for a given type of muscle, but it may vary over a wide range. This "contraction time" seems to be greatest in a fresh preparation

which has been inactive for some time. Our highest figure for the soleus under these conditions is 175 σ at 30°C. This same muscle, after many alternate periods of activity and rest, showed a value of 100 σ for the first contraction after a minute's rest, and of 50 σ for one of a series of partially fused twitches a minute after the beginning of stimulation at 9 per second. We have many records of twitches of fatigued soleus muscles which are scarcely 40 σ in duration from the first electrical disturbance to the maximum of tension. The duration of twitches of the gastrocnemius may shorten from 45 σ to 15 σ. Cooper and Eccles have already called attention to this phenomenon, but we also observe that in a fatigued muscle the duration is brief even though the total tension is slight. Therefore the higher total tension invoked by Cooper and Eccles in partial explanation of the phenomenon can hardly play a part in this case. The briefer time to maximum found in fatigue is not surprising, however, since if a smaller amount of energy is liberated by an impulse it should presumably expend itself in a briefer time.

We have already mentioned the two phases of relaxation, the first of which usually merges imperceptibly into the second, but sometimes, if it be slow and linear, gives way abruptly and forms a clear "nose" or "angle." The second phase is relatively constant but the first varies considerably in duration, rate of fall, and degree of curvature. The usual effect of fatigue is to slow this process and yield a more perfectly fused tetanus. If the circulation is good, however, the effect of activity may be actually to accelerate the first phase of relaxation. This is usually followed by a subsequent slowing.

The net result of these variations, which apparently depend upon a variety of factors which we have not undertaken to identify and which may aid or oppose one another in tending to produce a smooth tetanus, is to make the relation between fatigue level and frequency very uncertain except in its broad qualitative features. The difference between similar observations on a fresh muscle and on the same muscle after several periods of work, illustrated in figure 1, is an excellent example of this. Inspection of the original records shows the lower branch of the curve is due in this case to imperfect fusion consequent on a shortening of contraction time and acceleration of the first phase of relaxation.

The data for figures 1 and 2 were obtained by separate tests at each of the various frequencies. In other experiments the frequency was altered abruptly without allowing a cessation of activity. In such experiments if a soleus muscle which has been brought to its fatigue level with a rapid rate of stimulation, say 30 per second, is now stimulated at 15 per second, the resulting tension almost immediately begins to increase, as shown in figure 3. The tension rises to the value characteristic of the new rate of stimulation, reaching it in approximately half a minute. Suppose now that a

soleus muscle has first attained the level characteristic of 30 stimuli per second, and that the frequency of stimulation has then been reduced to 15 per second, and that the tension has increased to the new level. If, within a short time after the slowing of stimulation, when the new level has not quite or has only just been reached, the frequency is again increased, then the tension will drop again quite promptly to the first fatigue level. This fall in tension is not as abrupt as the relaxation when stimulation is discontinued entirely. In fact, if the muscle has been stimulated for some

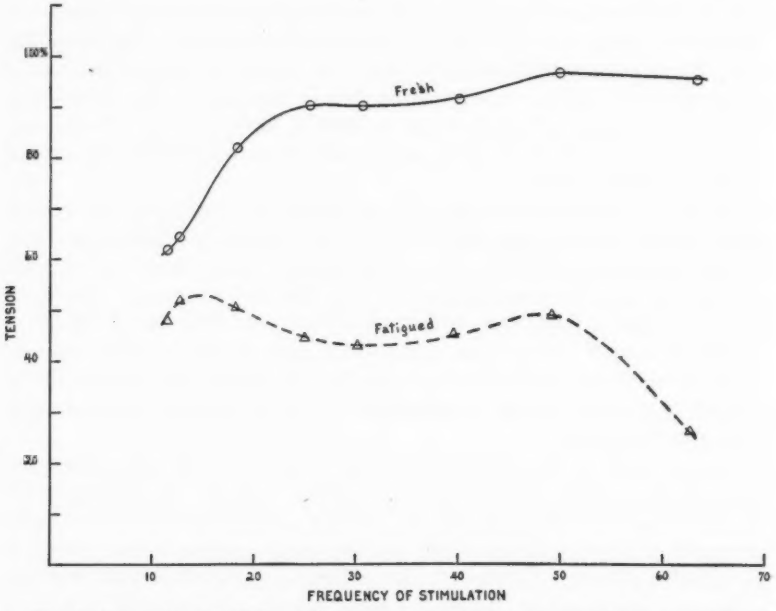

Fig. 2. November 16, 1931. Soleus muscle. Tension expressed as percentage of tension developed during test periods of 2 seconds' duration, stimulating at 62 per second. Observations made in random order. Blood pressure range from 110 to 145 mm. Hg. Other details as for figure 1.

time, half a minute or more, at the lower frequency, there is usually a transient rise in tension on speeding up the stimulation. This rise is only transient, being succeeded by the usual fall, and the greatest tension attained is never as great as that developed by a fresh muscle stimulated at the rapid rate in question. Even so, the rise in tension shows that the muscle after a period of stimulation at low frequency is nearer to the fresh state than when it is in the fatigued state characteristic of the high frequency. This leads to the paradoxical conclusion that after a muscle has been fatigued

at a high frequency of stimulation it can partially recover at a lower frequency *while maintaining a greater tension.* It should be noted that there is here no question of an incomplete tetanus in either case. The paradox can be achieved with rapid enough frequencies to give complete fusion throughout the experiment.

Action currents of the active muscle, recorded simultaneously with the contraction, offer a valuable clue to the solution of this paradox and to the nature of the maximum in the curve relating tension to frequency. Unfortunately the action currents as recorded are diphasic, since it was desired not to injure the muscle to render them monophasic, lest we interfere with the maintenance of tension. Changes in the height of the excursion of

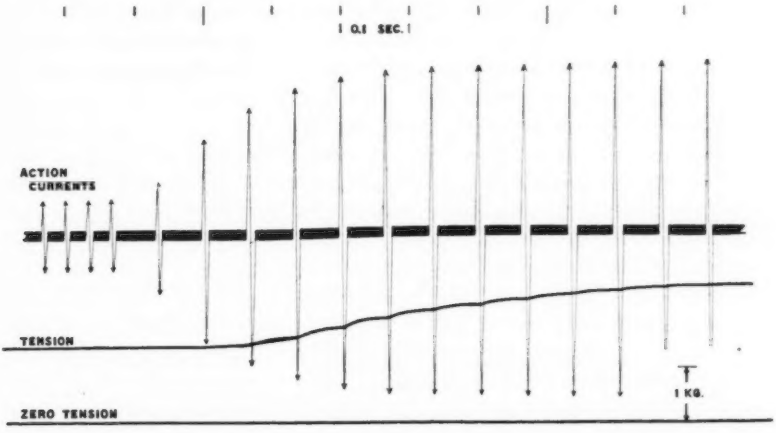

Fig. 3. March 26, 1929. Soleus muscle. Increase in tension and in height of action currents resulting from change in frequency of stimulation from 30 to 15 per second, redrawn to scale from original film. Stimulation at 30 per second had been in progress for 1 minute 40 seconds.

the galvanometer may therefore be due to some extent to a slowing of the rate of electrical change and an alteration of the time relations and relative strengths of the two phases; and indeed the action currents in the fatigued muscle show signs of all of these changes, which are already well recognized as characteristic of fatigue. There remains, however, a gross change in the size of the action currents, running closely parallel to the changes in tension, which it is impossible to explain by these minor alterations. We wish to emphasize this general correlation, which is illustrated in figure 4, rather than any detailed quantitative comparison.

The relationship between frequency of stimulation and the height of the first phase of the action current differs from the curve for tension,

since there is no question of fusion, as in the case of twitches, to complicate the case. Measurements of the action currents recorded about one second after the beginning of stimulation show only a slight depression at the highest frequencies employed. In the case of fatigued muscle, however, a depression with increasing frequency is very marked, as may be seen in figure 1. The very extreme depression, however, may be due in large measure to interference of the second phase of one action current with the first phase of the next.

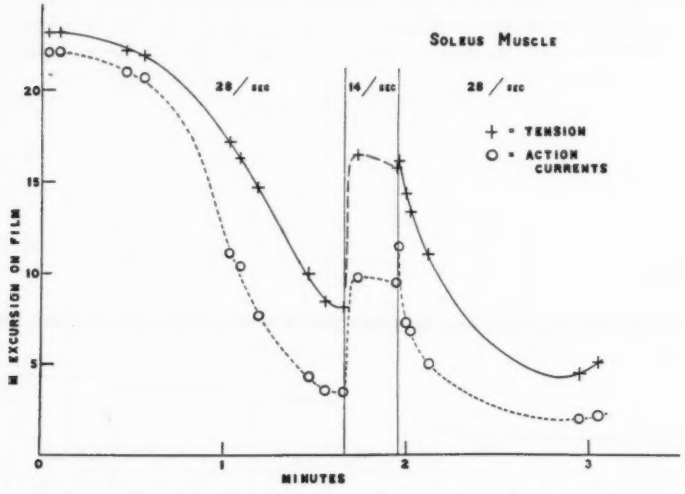

Fig. 4. March 26, 1929. Soleus muscle. Tension and action currents both measured arbitrarily in millimeters excursion on the film and plotted against duration of continuous stimulation. Vertical lines indicate moments of transition from frequency of 28 per second to 14 per second and reverse. Note the increase in both tension and action current on slowing the frequency and also the transient further increase on again stimulating at the higher frequency. Stimulation by rotary interrupter and induction coil. Make and break shocks effective.

In some experiments the action currents show a well-marked alternation in size, when the stimuli are relatively rapid and the muscle is fatigued. This indicates that some fibers are not responding to every stimulus. When the alternation appears it is usually quite regular and not by any means a random variation in the height of successive excursions. The most usual situation is that every second action current is five or ten per cent smaller than the ones preceding and following it. Differences of as much as twenty per cent are rare. Sometimes the pattern is more complicated although still regular, sequences of four action currents showing successive relative sizes in the order 1, 4, 2, 3 or 1, 3, 2, 4, where 1 indicates

the largest and 4 the smallest of the group. The change from one pattern to another is always gradual and never abrupt. In the majority of experiments, however, alternation of any sort is either very slight, absent, or endures for only a small part of the time, and is associated with the higher frequencies of stimulation.

The passage of alternate impulses through a region of partial local narcosis (or a fatigued neuromyal junction) is a familiar phenomenon (cf. Tsai, 1931), and the alternation may also arise at the stimulating electrodes (cf. Forbes and Rice, 1929), either because a rising threshold causes the make shocks to become ineffective in some fibers or because of anodal blocking. We do not believe that these latter effects were significant in our experiments because the stimulus was set well above maximal for make shocks at the beginning of the experiment, and, in nearly every case in which the test was made, increases and decreases of about 50 per cent in the strength of the stimulus caused no visible change in either tension or action currents, even in a fatigued preparation. We therefore ascribe the alternation to fatigue of the neuromyal junction.[2] The phenomenon is significant only as showing that some fibers in the muscle were not responding to every stimulus at the more rapid rates of stimulation, but rather were responding at only half the frequency. This in no way serves to explain the major phenomenon which is the diminution in tension which results from increasing the frequency of stimulation. On the contrary the effect would be to obscure this phenomenon. In fact we believe that the second maximum which sometimes appears in the tension-frequency curve for fatigued muscle at relatively high frequencies may be due in large part to alternation which results in some of the muscle fibers responding at a frequency only one-half that of the stimuli applied to the nerve. These fibers stimulated at a lower frequency would be expected to develop relatively more tension than those which are responding to every stimulus. It is probably more than a coincidence that, when present, the second maximum usually appears at double the frequency of the main one.

Diminution of activity associated with a higher frequency of stimulation immediately suggests the phenomenon of "Wedensky inhibition" (cf. Forbes, 1922) as a possible explanation. In this case we would suppose that, as fatigue develops, the relative refractory period of the nerve or neuromyal junction becomes so prolonged that the tissue does not have time to recover completely between successive impulses at the higher frequency. The impulses will therefore be subnormal in size and may become so small as to fail to excite the muscle fiber. At the lower frequency of stimulation, however, recovery would be almost or quite complete after

[2] The alternation can hardly have been dependent upon irregular spacing of the stimuli, since no differences in the intervals between the action currents can be detected, and alternation was not an invariable phenomenon.

each impulse and the impulses would be large enough to excite the muscle. This interpretation would imply that the low tension developed under rapid stimulation is due to the blocking of impulses in some of the neuromyal junctions and the relaxation of the corresponding muscle fibers. The maintenance of the tension which does persist might be explained on the basis of alternation of activity among various motor units. In any case the "Wedensky" interpretation implies that a large proportion of the muscle fibers become completely inactive.

This explanation is inadequate in the present case, although we cannot rule it out absolutely as a minor contributing factor. We sought experimentally for inactive fibers in the muscle by applying supramaximal induction shocks directly to the muscle during its tetanic contraction. For this purpose the galvanometer leads were transferred to an inductorium by means of a double-throw switch. Since the contraction of the muscle is virtually isometric, the resulting twitch in any idle fibers should add almost quantitatively to the preëxisting tension and be easily detected. The result of this test was negative. In a typical experiment a soleus muscle was fatigued by stimulation at the rate of 30 per second until the tension was 35 per cent of its initial value of 2250 grams. Slowing the stimulation to 15 per second resulted in an increase of tension to 71 per cent of the initial maximum. This transition is illustrated in figure 3. Again speeding up the stimulation, the tension fell eventually to 20 per cent. Intercurrent direct stimuli now caused increases of tension of 77 grams, as compared with 530 grams developed in response to the same stimulation 3 seconds after the cessation of the tetanic stimulation. Therefore at most only 14.5 per cent of the fibers could have been idle during the tetanic stimulation. This will not explain the diminution in tension under the high frequency of stimulation, or even the difference in tension exhibited by the two frequencies. Probably even fewer fibers are idle than is suggested by the above figures, since there is an alternative explanation for the increment of tension in response to direct stimulation which will be considered below.

Further evidence against the Wedensky phenomenon is found in the behavior of the action currents and tension immediately after the slowing of the rate of stimulation. The shift of frequencies is absolutely abrupt, as shown in figure 3. The resulting change in action currents and tension is gradual. If we were dealing with a Wedensky inhibition, however, the first impulse to arrive after the longer interval should be much larger than those at the higher frequency and should at once excite a large number of idle fibers. We should expect a sudden increase in both tension and action currents. The only alternative is to assume that a still further increase in the size of the nerve impulses due to "equilibration" (Forbes and Rice, 1929), is necessary to bring them to a strength adequate to excite the idle

fibers; but this possibility seems remote. In fact, the very regular gradual increase in the size of the action currents of the muscle strongly suggests that we are dealing rather with a process of "equilibration" in the muscle itself.

A few observations have been made in which the activity of the muscle has been broken up into alternate periods of half a second of contraction and half a second of rest. The frequencies of stimulation, as in most of the experiments on sustained contraction, were 30 and 15 per second for the soleus and 50 and 25 for the gastrocnemius. The results were essentially similar for the two muscles, and a description of one will suffice. The gastrocnemius, stimulated in this fashion, responds with a series of tetanic contractions which at first continue to increase in tension throughout the entire half-second of stimulation. In the course of about half a minute the contractions each show a level plateau, the maximum being reached in about a fifth of a second. Then, by degrees, more rapidly with the more rapid rate of stimulation, the tension begins to fall off during the latter part of each tetanus, until ultimately we find a brisk initial twitch, rising to perhaps 50 per cent of the tension developed by the fresh muscle, followed by a rather abrupt drop to a definite plateau which is maintained for the remainder of the half-second of activity. This plateau is lower and the initial twitch is higher for the rapid stimulation than for the slower. The level of these plateaus becomes essentially constant for a given frequency and obviously corresponds to the "fatigue level" attained with continuous stimulation. If the frequency of stimulation be changed there is a gradual shift to the picture characteristic of the new rate of stimulation, the twitch and the plateau being modified in opposite directions.

The action currents in such an experiment correspond closely in size to the tension developed by the muscle. When the fatigued state is reached, the action currents at the beginning of each brief tetanus are large, comparing favorably with those recorded from the fresh muscle. They rapidly shrink to a much smaller size, corresponding to the low plateau which terminates each tetanus. These findings are illustrated in figure 5. The interesting features of this experiment are the rapid recovery which the muscle makes during each half-second of rest, and the inability of the muscle to maintain this recovered state. The benefits of the rest are intense but fleeting. Half a dozen impulses suffice to bring the muscle back to the fatigue level. The situation recalls that described by Matthews (1931) in the recovery from adaptation of the sensory end-organ in the muscle of a frog.

Discussion. The curve relating the tension developed in brief tetani to the frequency of stimulation can probably be explained entirely as the result of more or less perfect fusion of the individual twitches. For the tension maintained at the "fatigue level" the same principle serves to

explain the rising branch of the curve at low frequencies of stimulation. As is already well known, fusion generally occurs at a lower frequency in the fatigued than in fresh muscle.

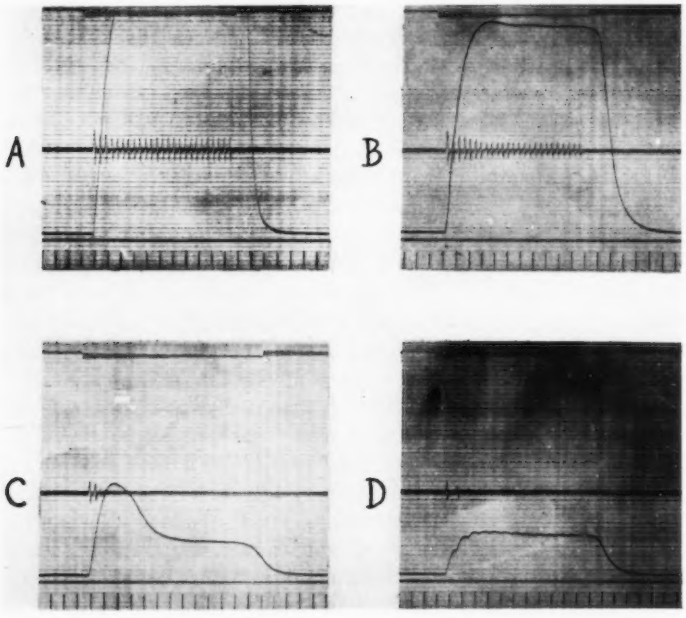

Fig. 5. December 14, 1928. Gastrocnemius muscle. Intermittent stimulation for periods of ½ second separated by ½ second rest.

A. Second period of stimulation. Tension record goes off film. The second recording fiber referred to in text was not in use during this experiment.

B. 24th period of stimulation, showing slightly reduced tension and action currents.

C. 45th period, showing initial hump falling to a much lower plateau. Action currents during plateau are barely discernible on original film.

D. During the half second period following C frequency of stimulation was slowed from 52 to 26. This record shows the third period of stimulation at the reduced frequency. The initial hump is absent but the plateau is higher than in C.

Time interval at bottom equals $\frac{1}{25}$ second. Next line is line of zero tension.

A second factor must also come into play to diminish the effectiveness of rapid stimulation on fatigued muscle. It is apparent that when the frequency of stimulation surpasses a certain rate, the energy liberated per impulse must be reduced, even in the case of the fresh muscle, for otherwise the efficiency of the muscle in terms of the H/T ratio would be dimin-

ished in inverse ratio to the rate of stimulation. No such diminution was observed by Bronk (1930) in excised muscle, but unfortunately his observations were confined to tetani of only 2 seconds' duration or else to a single slow rate of stimulation. Neither do Visscher and Smith's (1930) observations touch the point, as their frequencies of stimulation were so slow as to yield practically discrete twitches. Any such extreme lowering of efficiency seems unlikely, however. The diminished action currents also indicate strongly that the energy released by each impulse is reduced. This follows qualitatively from the approximately parallel variations in action current and tension, whether we regard the energy liberation as governed by the process which yields the action current, or whether the action current results from the liberation of energy for the contraction. The reduced tension resulting from more rapid stimulation is then an expression of a greater reduction in the energy liberated per impulse than can be offset by the greater frequency of the impulses.[3]

Such a reduction associated with rapid frequencies of response immediately suggests the familiar refractory period of nerve and muscle. It is well known that the action current of both types of tissue is reduced in the refractory period, and also that the refractory period is prolonged by continued activity. The "equilibration" of Gerard (1927) must also be considered. We have a clear case of "equilibration" in the reduction observed in the size of the action currents during the first few seconds of stimulation. They fall to a level which is lower the more rapid the rate of stimulation. Whether, as in nerve (Forbes and Rice, 1929), the further fall in action currents to the fatigue level is due to a further progress of equilibration as opposed to a lengthening of the refractory period, we have not attempted to determine. The distinction is of no consequence in the present argument. If we assume that the liberation of energy causing contraction is determined in amount, other things being equal, by the process which underlies the action current, or directly by the action current itself, we have a satisfactory explanation of the phenomena. In favor of the latter suggestion we have the familiar ability of an electric current to produce a persistent local (cathodal) contraction independent of a propagated disturbance (Lucas, 1907). It is on this basis that we are inclined to interpret the slight increments of tension yielded by an already active but fatigued muscle in response to intercurrent direct stimuli (see p. 350 above). There is also a considerable amount of evidence (Fulton, 1925a, b, 1926) that action currents and contractile tension vary *pari passu* in a variety of conditions.

This hypothesis is not necessarily refuted by the observations of Bogue and Mendez (1930) who state, in regard to cardiac muscle, that "there is

[3] This statement must be modified slightly if the efficiency of the muscle is significantly reduced under these conditions.

no relation between variations in the height of the initial (electrical) deflection and in the force of contraction." It is obvious that even if the initial phase of the action current of muscle serves to initiate the chemical changes resulting ultimately in contraction, contraction might still be impaired by conditions affecting the contractile mechanism which might leave the conducting and excitatory mechanisms unaltered. The effects of calcium and of acetylcholine (Bogue and Mendez) and of water (Bishop and Gilson, 1927) we must tentatively interpret in this way. As Bogue and Mendez significantly remark "on the other hand, there is no evidence that the height of the electrical response can be reduced without affecting that of the mechanical responses."

In supposing that the action current is the mechanism which initiates and, other things being equal, determines the extent of the chemical processes resulting ultimately in the development of tension, we refer only to the "initial spike" of the muscular action current described by Bishop and Gilson (1927). The subsequent plateau which they also describe apparently depends upon the chemical or physical events in the contractile mechanism.

If our hypothesis is correct, the problems of the maintenance of tension in a muscle and of its response to various frequencies of stimulation become in part a problem of a process of "equilibration" or adaptation of the excitatory and conducting mechanism. We may expect the general principles deduced for nerve (cf. Gerard, 1927) to apply likewise to this situation. For example, deepening the ether anesthesia depresses markedly the tension developed by fatigued muscle. This is readily explained in terms of the recognized effect of ether in prolonging the refractory period of excitable tissues (Kato, 1924; Tsai, 1931).

It should be evident that the factor which prevents a muscle fatigued under rapid stimulation from developing as much tension as when fatigued under slower stimulation is not an exhaustion of the available store of fuel (e.g., glycogen) or the rate at which oxygen is supplied to it, since appropriate slowing of the rate usually causes an immediate increase in tension. Evidence has been presented to show that the effect is not due to a failure of the individual fibers to respond. The limiting factor must therefore lie in the mechanism determining the amount of energy released by each excitatory impulse. This does not mean, however, that the performance of a muscle under continued stimulation is independent of the blood flow through it. Dr. C. E. Leese is accumulating evidence on this aspect of the question, and it appears that if the blood supply is inadequate, the tension of the fatigued muscle will fall to a very low level practically independent of the frequency of stimulation. Such a relationship in no way affects our interpretation, since the tension developed by a muscle may obviously be reduced by various independent factors. Too high a frequency of stimulation is only one of these.

We may emphasize again the relatively low rates of stimulation which are most effective for sustained contraction. Our results are completely in accord with those of Briscoe (1931), and we believe that our interpretation is adequate to explain all of her phenomena. The rates which we find effective for sustained contraction agree well with those directly observed in the reflex discharge governing postural tone (Adrian and Bronk, 1929).

SUMMARY

When a circulated soleus nerve-muscle preparation of a cat is stimulated for two or three minutes, the tension developed by the muscle falls from its initial value to a lower level which is then maintained with little or no further change for many minutes. The gastrocnemius muscle under these conditions sometimes does not quite maintain a steady state, and at best maintains only a smaller fraction of its initial tension than does the soleus.

The tension maintained in a muscle after three minutes of stimulation is a function, among other things, of the frequency of stimulation. If the condition of the preparation is good, the curve relating the tension exerted by the fatigued muscle to frequency shows a maximum for the soleus between 15 and 20 stimuli per second. For the gastrocnemius the corresponding frequency is between 30 and 50 per second.

The low "fatigue level" associated with high frequencies of stimulation is not due to a "Wedensky inhibition."

A muscle brought to a low "fatigue level" by continued stimulation at high frequency will partially recover if stimulated at a slower rate. The tension rises to approach that characteristic of the new rate of stimulation. On returning to the more rapid rate there is usually a transient further increase in tension, indicating that the muscle has partially recovered even while exerting the greater tension.

When the tension developed within the first second of stimulation is plotted against frequency of stimulation, the plotted curve rises with increasing frequency along an S curve up to at least 60 per second for soleus and 120 per second for gastrocnemius.

Fusion of twitches into a smooth tetanus usually occurs at a lower frequency in the fatigued than in the fresh muscle. This depends upon a slower rate of relaxation which is one factor favoring the maintenance of relatively large tensions at low rates of stimulation.

The first spike of the (diphasic) action current of the muscle after the "fatigue level" has been reached is smaller the more rapid the rate of stimulation.

If, in a fatigued preparation, the frequency of stimulation is abruptly altered, keeping it above the limit required for fusion of twitches, tension and action currents increase or decrease in parallel, approaching their new levels asymptotically.

Cessation of stimulation for half a second in a fatigued muscle results in a considerable but transient increase in both tension and action current when stimulation is resumed.

An explanation of these phenomena is offered, involving the hypothesis that under normal conditions the action current or the process underlying it determines quantitatively the amount of energy made available for the development of tension. The tension exerted by a fatigued muscle at a given frequency of stimulation is thus a resultant of the "equilibration level" of the conducting mechanism and the rate of relaxation of the individual twitches.

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STUDIES ON ALBUMINURIA FOLLOWING EXERCISE

I. Its Incidence in Women and its Relationship to the Negative Phase in Pulse Pressure

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White clouds in the urine had been noted for centuries before Richard Bright interpreted the significance of this finding and published his epoch-making description of essential nephritis. Fifty years passed before clinical men began to examine the urine of the apparently healthy and it became known that albuminuria might occur unassociated with the signs and symptoms of nephritis. Protein in the urine was found to be a frequent concomitant of pubescence and an inferior constitutional build. Pavy (1886) early pointed out the peculiar relationship of the upright position to this anomaly of adolescence, and Stirling (1887) suggested the name "postural albuminuria." Subjects who suffer from this albuminuria frequently have an exaggerated lumbar lordosis, and Rieser and Rieser (1922) presented the hypothesis that this postural defect mechanically interferes with renal circulation, producing a stasis which makes the kidney permeable to albumin. Erlanger and Hooker (1904) made an extensive study of a case of orthostatic albuminuria and found that the diminution in pulse pressure with the assumption of the upright posture was the only factor consistently related to the output of albumin. Dunhill and Patterson had already observed that albuminuria might follow upon exercise, and Lowsley (1911), studying the cardiovascular responses to various types of physical activity, noted that post-exercise albuminuria was associated with the occurrence of a subnormal drop in pulse pressure.

The general opinion among clinicians and laboratory investigators has been that these types of albuminuria are benign, being based upon no permanent underlying renal disease; such kidneys are generally considered functionally as competent as the impermeable kidney. To call such intermittent albuminuria functional implies it to be a physiological response. The evidence of the benign nature of these albuminurias is not conclusive merely because they are not the obvious precursors of nephritis. They should not be dismissed before their cause has been more perfectly elucidated. Few studies have concerned themselves with the incidence of post-

exercise albuminuria in the female. The purpose of this study is to determine the frequency with which it occurs after experimental exercise in women, and to study its relationship to the post-exercise fall in pulse pressure.

Incidence of post-exercise albuminuria. From 47 individuals, analyses were made of the urine collected immediately following experimental bouts of physical exertion of various types, such as riding the bicycle ergometer, running around an indoor track at top speed, and working in a rowing machine. Of the subjects studied, 14.8 per cent showed albumin in a sample of urine voided before the onset of exercise. This pre-exercise albumin did not always increase in quantity after physical exertion. Once it remained unchanged and three times reduced in amount. Of those who had no albumin in the urine before exercise, physical work produced an albuminuria in 57.5 per cent and its severity was inversely proportional to its frequency of occurrence.

Relationship of post-exercise albuminuria to the negative phase. In this laboratory Henry and Dawson (Henry, 1928) had investigated the negative phase following work on a bicycle ergometer and had confirmed the observations of Lowsley (1911) who found that there is a marked elevation of pulse pressure during exercise, followed by a diminution to a level below the pre-exercise normal after the cessation of muscular activity. This period of subnormal pulse pressure has been termed the negative phase. The bicycle ergometer permits a bout of exercise that can be readily adjusted in severity to throw the subject into a post-exercise condition with a cardiovascular reaction characterized by a diminution in the amplitude of the pulse pressure.

The standard dosage of work was a half-hour ride on a bicycle ergometer with a Prony brake, the tension on the brake band being kept constant at three pounds. The subjects adjusted the severity of the work to their own fitness by riding steadily at individual maximal speed. Observations were made upon twenty-three subjects. Those upon whom the primary work of the study was conducted were young women physical education students, familiar with physiological experimental technique and coöperative to an unusual degree. As a whole the group might be characterized as one accustomed beyond the ordinary to strenuous physical exertion.

The primary object of these experiments was to determine whether or not post-exercise albuminuria is produced by a diminution in pulse pressure to a level below normal. It was necessary to obtain three urine samples: first, a urine sample before the onset of exercise as a standard for comparison; second, a urine sample immediately at the cessation of exercise to determine whether or not the kidney becomes permeable to protein during the period of active waste production and the phase of hypertension with high pulse pressure; third, a sample of urine produced by the kidney during the

post-exercise period of negative phase when the pulse pressure sinks subnormally.

There was frequent difficulty in the collection of urine samples, both before the bicycle ride and particularly, immediately after. The first of these was a psychic inability to void, which disappeared spontaneously after the subject became familiar with the experimental procedure. It was difficult to determine whether the inability to collect urine samples after exercise was also psychic or due to an exercise anuria. Following the suggestion of the procedure routinely adopted when the phenolsulphonephthalein test of kidney function (Todd, 1925) is given, the subject drank 200 cc. of water after she had collected her first sample of urine and approximately one-half hour before the onset of the ride. This sufficiently promoted kidney secretion and henceforth in the majority of cases samples immediately after exercise were successfully collected.

The details of the standard technique follow: The subject came to the laboratory and changed into riding clothes. Urine sample 1 was collected, the bladder being completely emptied. Two hundred cubic centimeters of water were taken by mouth. After cardiovascular stabilization had occurred, systolic and diastolic pressures were determined, and the pre-exercise pulse pressure was recorded. The subject began to ride the bicycle ergometer twenty to thirty minutes after the intake of the water. When the subject became heated, an electric fan was turned on and subsequently the laboratory windows were widely opened to insure a sufficiently low room temperature and a free circulation of air. The subject rode for one-half hour. Immediately at the cessation of the ride, urine sample 2 was collected, the bladder being again completely emptied. The subject drank a second 200 cc. of water and was well covered to prevent chilling. Blood pressure readings were commenced and the time of onset of the negative phase was recorded. Readings were usually continued until the pulse pressure returned to normal, the subject remaining quietly seated throughout this procedure. When the negative phase was protracted, urine sample 3 was collected before the return of the pulse pressure to normal, since the period was sufficiently long to permit the kidney to secrete an adequate sample of urine.

The urine samples were tested for albumin by two methods: heat and 3 per cent acetic acid, and by salicylsulphonic acid. McNabb and Field (1924) had found considerable difference in the sensitivity of the various urinary albumin tests in common use by clinical laboratory diagnosticians. The heat and acetic acid test was, in their hands, the most satisfactory. They found the salicylsulphonic acid test next to heat and acetic acid in its delicacy. The technique suggested by McLean (1924) was used. Six drops of a saturated aqueous solution of salicylsulphonic acid were layered upon a half inch of urine in a test tube. Without heating, large amounts

of albumin immediately formed a dense white precipitate, while small amounts produced a definite milkiness or an easily detectable opalescence.

There were twenty-five experiments with records of both blood pressure and urinalysis findings. Of these, five were eliminated from the pulse pressure study because the subjects showed albumin in urine sample 1 collected before the onset of exercise. The results of five more were cast out because the subjects were unable to obtain urine sample 2. Several of these were contributed by one subject, an experienced laboratory worker who was never able to void immediately after the cessation of the exercise. One was eliminated because albuminuria appeared immediately after the ride. This phenomenon was subsequently studied and is discussed in the second paper.

The results may be summarized as follows: *a.* Eight experiments demonstrated a fall in pulse pressure unassociated with albuminuria; the average pulse pressure drop was 11.2 mm. of mercury below normal, and the average duration of the negative phase was 51 minutes. *b.* Six presented a fall in pulse pressure with a concomitant albuminous urine, confined solely to urine sample 3, secreted during the negative phase; the average pulse pressure drop was 21.3 mm. of mercury below normal and the average duration of the negative phase was 72 minutes. This is a diminution 90.1 per cent greater than the depth of the negative phase and 41.8 per cent longer than that occurring in the group whose kidneys did not become permeable to protein.

To confirm the evidence of an etiological relationship between post-exercise albuminuria and the negative phase, a subject was trained to void at short intervals of time without change in position and cardiovascular disturbance. To aid in the secretion of adequate samples, 50 cc. of water were administered at constant intervals, at the time of the collection of urine samples. All urine samples were centrifuged and tested quantitatively for albumin, using the sulphosalicylic acid turbidimetric method of Kingsbury, Clark, Williams and Post (1926).

The subject was well stabilized in the sitting posture. The bladder was then completely emptied and with the administration of the first 50 cc. of water, observations were commenced, blood pressure readings being made at sixty second intervals and urine samples collected every twenty minutes. Pre-exercise blood pressure was observed during the first twenty minute experimental period. After voiding the pre-exercise urine sample, the subject rode the bicycle ergometer for twenty minutes, doing a moderately severe piece of work at a pedalling rate which averaged 80 revolutions per minute. Immediately at the cessation of the ride, the exercise urine sample was voided and blood pressure readings were resumed. Systolic and diastolic pressure fell steadily and there was a very moderate reduction in pulse pressure during the first twenty-two minutes after the exercise. The

diastolic pressure then began to drop precipitously and a less acute fall in systolic pressure occurred. This terminated in a syncopal attack. Blood pressure readings were resumed with the subject in the recumbent position. The subject slept during the succeeding hour, being aroused periodically for the collection of urine samples. The pulse pressure fell gradually and then slowly approached the pre-exercise level. After the return trend had been established, the subject was again placed in the sitting posure. A secondary pulse pressure drop occurred with the assumption of the vertical position. Observations ceased three hours after the termination of the ergometer ride.

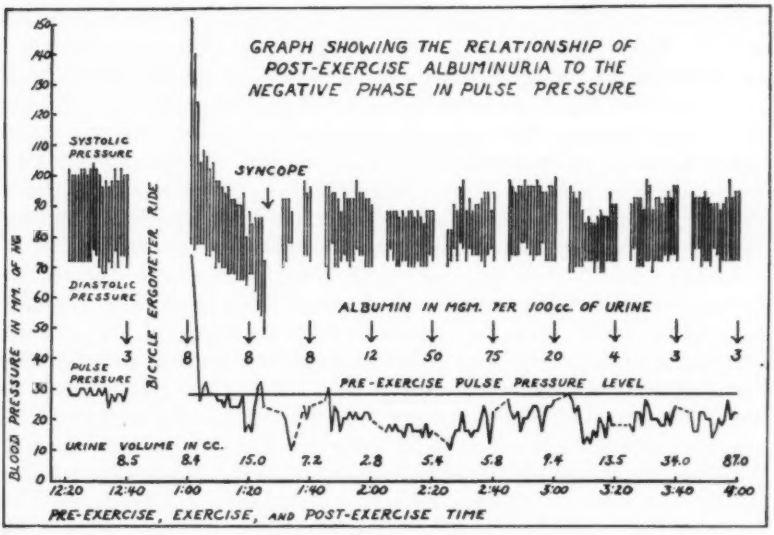

Fig. 1

The accompanying graph shows the pre- and post-exercise cardiovascular reactions, the amount of urine secreted and the quantity of albumin produced during each twenty minute period. The greatest amount of albumin appears during the period of most marked oliguria and the maximal reduction in the amplitude of the pulse pressure.

These findings, which indicate a relationship between the negative phase and albuminuria, may possibly be explained by suggestions that come from recent work on the physiology of the kidney. In view of his observations on spontaneous interruptions occurring in the circulation of individual glomeruli (Richards and Schmidt, 1924), Richards (1925) believes that when a constriction stimulus reaches the arteriole and produces its partial clo-

sure, the wall of the vessel suffers from an oxygen deficiency which depresses it directly. The cells of the tubules surrounding the affected arteriole are subsequently also subjected to anoxemic conditions. This oxygen deficiency institutes the production of substances which have a vaso-dilator effect. The period of intermittence in any single glomerulus is, under normal conditions, so short that there is no anoxemic damage to the endothelium of the glomerulus and no albumin appears in the urine. Richards suggests that albumin might thus result under conditions not far removed from the physiological, when for any reason the duration of the intermittent cessation of glomerular flow increases. Many years ago, Herrmann (quoted by Fischer, 1912) had shown that local circulatory disturbances of the kidney produce an albuminuria, and the later experiments of Araki and of Zillessen (Fischer, 1912) demonstrated that a diminution of the oxygen supply to any parenchymatous organ, through interference with its normal circulation, is followed by a localized accumulation of acids. Wearn and Richards (1924) introduced a quartz needle into Bowman's capsule and removed urine secreted by the glomerulus. They observed that when the blood flow through the glomerular capillaries was rapid, there was no albumin in the urine secreted but that when the circulation was sluggish and the capillaries were dilated, the urine became albuminous.

During exercise there is a marked elevation of the pulse pressure, an increase in the circulation rate, and a shunting of blood to the active muscles and the skin. Muscular exercise elevates the level of the metabolism and concomitantly the production of heat. If the exercise is severe enough the production of heat may exceed the heat loss and the temperature may rise. When the bicycle ergometer was ridden with a heavy load at a high speed for as short a time as two minutes, the body temperature elevated 0.1° to 0.4°F. during nine out of ten consecutive rides. There is normally a balance between the functional activity of the kidneys and the skin. The kidneys become relatively inactive when large amounts of water are being lost through the skin, one of the most efficacious of the channels of heat loss. Some of the subjects remained pale throughout the course of the half hour ride and experienced little sweating. Others became flushed, with an irregularly distributed dull red blotching, most commonly confined to the face, arms and thighs. The subjects in whom visible perspiration was profuse exhibited marked dilatation of the peripheral vessels in the skin, and it was observed that the incidence of post-exercise albuminuria was greatest in the cases with such peripheral change. The shunting of large volumes of blood to the skin in addition to the active muscles in the extremities reduces the flow of blood through the visceral area. Those in whom the peripheral dilatation is excessive have shunted more blood away from the kidneys and under these circumstances, the intermittence of glomerular circulation may persist beyond the limits of normal and produce an asphyxial damage of the kidney.

After exercise ceases, temperature equilibrium is reëstablished at a lower level and cardiovascular recovery takes place. The systolic pressure falls rapidly to a point below normal. The diastolic pressure is slower to fall and the pulse pressure drops. There is frequently a secondary rise in diastolic pressure coincident with the maximum pulse pressure fall. The circulation in the kidneys is sluggish until sufficient time elapses for the reëstablishment of a proper balance between the systolic and diastolic pressures. Acids accumulate in the cells of the kidney, and when the glomeruli open, blood proteins escape.

Two causes therefore may combine to permit a leakage of albumin through the glomerular endothelium; first, asphyxiation during the exercise; second, sluggish circulation after the exercise. In those subjects in whom there occurs excessive peripheral dilatation, and in whom a deep and protracted fall in pulse pressure follows upon the cessation of exercise, we find the highest incidence of post-exercise albuminuria.

SUMMARY

1. The incidence of albuminuria increased from 14.8 per cent before exercise to 57.5 per cent after exercise.

2. The pulse pressure fell below normal in all cases after a half-hour bicycle ergometer ride.

3. The pulse pressure fall in the cases with post-exercise albuminuria was 90.1 per cent greater in depth and was maintained 41.8 per cent longer than the pulse pressure fall in the cases without albumin following physical exertion.

4. The greatest amount of albumin was found to occur during the period of oliguria and the maximal reduction in the amplitude of the pulse pressure.

5. The experimental results may be explained by the hypothesis that strenuous exercise shunts the blood to the working muscles and the skin, affecting the circulation of the kidney in such a way as to cause asphyxiation of the renal cells beyond that compatible with normal function. The deep and protracted post-exercise low pulse pressure adds to the anoxemia already present and induces an abnormal accumulation of acids in the renal tissue which alters its permeability to the blood proteins and albumin appears in the urine.

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STUDIES ON ALBUMINURIA FOLLOWING EXERCISE

II. Its Relationship to the Speed of Doing Work

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In the course of the studies concerning post-exercise albuminuria in its relationship to the negative phase in pulse pressure, it was observed that when the exercise was severe, exhausting and carried on with intermittent bursts of speed, albumin appeared in the urine immediately after exercise and there was an apparent reduction in its amount during the subsequent period of low pulse pressure. The albuminuria following moderate, prolonged and steady exercise had been confined solely to the period of low pulse pressure. The purpose of this study is to ascertain whether there is any relationship between albuminuria and the speed of doing work.

The augmentation of the metabolic activities of the tissues, the physicochemical changes in the blood and the magnitude of the cardiovascular responses to exercise are all related to the speed, duration and severity of the muscular exertion. They may be used as indices of the cost of the effect achieved for they bear a relationship to the effort put forth. Post-exercise albuminuria may be a physiological response or it may be the concomitant of exercise too severe to be tolerated without pathological change. The presence of albumin in the urine or the failure of post-exercise albuminuria to occur cannot be evaluated without quantitative data concerning the work done.

In a physical sense time is not a factor which enters into the estimation of work, but physiologically it is as important to know with accuracy the rate at which work is being done as to know the work accomplished. In practice the Prony brake ergometer was found unsatisfactory for accurate quantitative estimations of the rate of working. The mechanical power input of the Prony brake is transformed by friction into heat and dissipated. Its coefficient of friction varied so markedly during any given run that the instrument could not be used without frequent load adjustment and the constant assistance of an operator. A new bicycle ergometer was therefore developed on the electrodynamic brake principle.

In the electrodynamic brake bicycle ergometer the mechanical power of

pedalling is transformed into electrical power which in turn is converted into heat and dissipated into the surrounding medium. The fields of a small dynamo are excited with a constant current from a storage battery, generating a flux which is cut by the conductors on an armature rotated by the movements of the bicycle pedals. This cutting of the flux generates a voltage which bears a fixed relationship to the rate of pedalling.

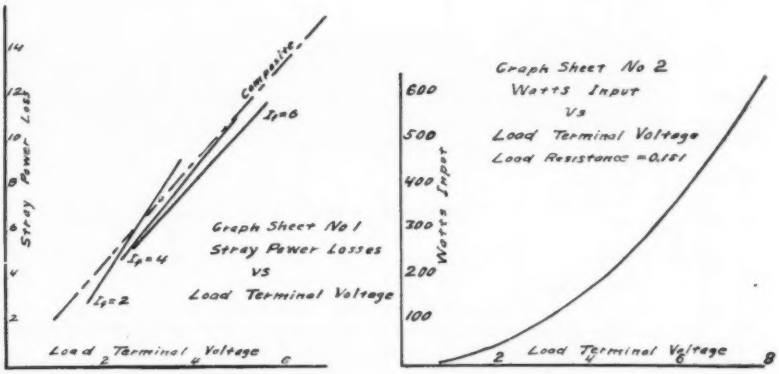
A standard woman's bicycle frame was firmly fixed by metal braces to a stout base-board. A twelve volt Dodge motor starter was mounted under the steering fork and arranged to be driven directly by the pedals through racing sprockets having 80 and 14 teeth, giving a gear ratio of 1 to 5.71. The load is the electric generator. A cast-iron fly-wheel was attached to the armature. The generator was connected for separate excitation of the shunt field, the series field having been disconnected. The exciting current was furnished by storage batteries and controlled by a sliding-contact rheostat placed within easy reach of the subject. The field current was indicated on a distant reading ammeter mounted on an upright and placed directly in front of the subject. The generator was loaded on a fixed resistance of 0.151 ohm, the value of which is independent of temperature. A distant reading voltmeter was connected across the load resistance and mounted on the upright above the ammeter. The voltmeter reading for any field current is controlled by the speed of pedalling. The magnitude of the load and the rate of pedalling are both indicated on the voltmeter. The ammeter gives the relation between the magnitude of the load and the rate of pedalling.

The mechanical input of the ergometer is used up in overcoming the rotative losses and in supplying the power generated in the armature. The rotative losses are made up of windage, the mechanical friction of the bearings and the chain, and the eddy current and hysteresis losses. They are determined by the electrical input method. The generator is run as a motor at various speeds and field currents and at no load. The stray power losses are practically independent of the load and vary only slightly with the different field currents and speeds. Graph 1 shows the stray power losses plotted against the generated voltage. The curves for the different field currents nearly coincide. Since they vary almost as the generated voltage, the rotative losses are considered as proportional to this voltage. The error incurred in estimating them as such is negligible.

The generated power is the product of the armature current and the generated voltage. It is the delivered power plus the armature copper loss. The copper loss is computed from the armature resistance and the current. A simple equation has been developed for the calculation of the generated power in terms of the machine constants and the terminal voltage; to this the rotative losses are added and the total input is obtained. This is a function of the load terminal voltage. Since the load resistance

is constant, the armature current is proportional to the voltmeter reading; since for any field current, the rate of pedalling is also practically proportional to the voltmeter reading, the speed and load will be constant for any fixed field current and voltmeter reading. Graph 2 is a calibration curve for the ergometer. The load in watts for any voltmeter reading is not affected by the field current and therefore the curve is independent of the ammeter reading. It shows the total watts input as a function of the voltmeter reading. For the same power input a change in excitation will modify the rate of pedalling. If the pedalling is to be fast, the field current is made low. A high field current requires a low rate of pedalling for the same power input.

In actual operation the subject sets the field current at an assigned value and rides at such a rate that the voltage is held constant at another assigned

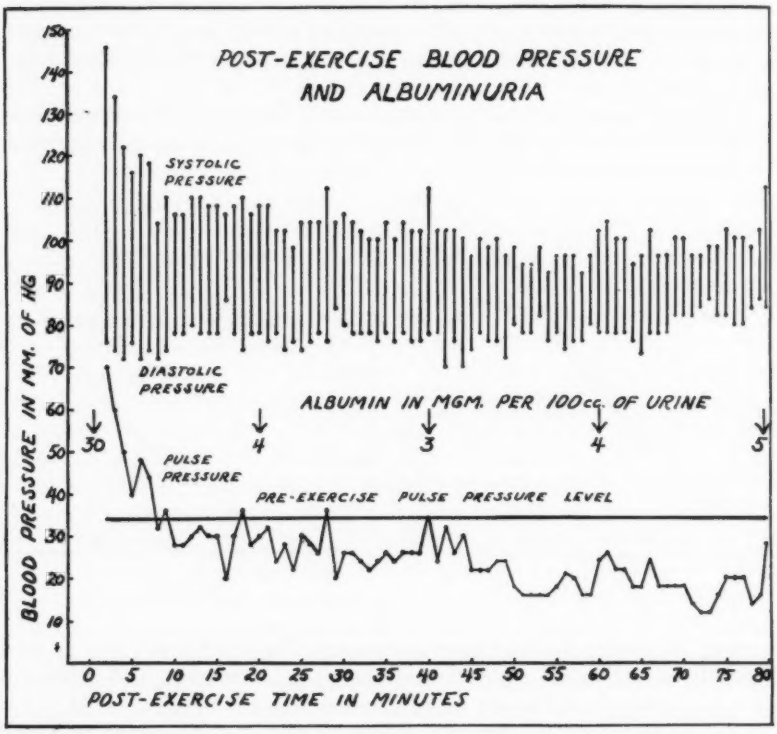


Graph 1 Graph 2

value. Knowing the load terminal voltage, the watts input may be read directly off the graph which accompanies the instrument, and if desired, converted into horse power or kilogram-meters per unit time. It is not sufficient to keep the average speed constant; the instantaneous speed must be unchanging. If two runs are made at the same *average* speed but with different *instantaneous* speed, the average powers may not be alike. The voltage must be kept constant. In practice it varied slightly but because of the fly-wheel effect on the armature shaft, the inexperienced subject was observed to closely approximate the steady pedalling of the experienced rider. The average speed of pedalling may be checked by recording the total revolutions of the armature shaft from ride to ride. The apparatus permits a bout of exercise to be repeated with very great accuracy any number of times, and the power input may be easily deter-

mined. It adapts itself especially well to the repetition of equivalent amounts of work at varying speeds.

The time consumed in doing work modifies greatly its cost and it is common knowledge that it is more expensive in terms of energy input to go fast than slow. As noted, it had been observed that long and violent rides carried on with intermittent bursts of speed were associated with an immediate post-exercise albuminuria. Graph 3 shows the cardiovascular



Graph 3. Showing an immediate post-exercise albuminuria and the increase in albumin during the negative phase in pulse pressure.

response to such a bout of exercise. The subject was flushed, dyspneic, and exhausted at the termination of 25 minutes of pedalling. A urine sample voided immediately after the cessation of work contained 30 mgm. of albumin per 100 cc. The subject voided at 20 minute intervals. The first two samples collected showed a marked reduction in the amount of albumin present. The post-exercise cardiovascular response was typical, the pulse pressure diminishing in amplitude to a value below the pre-

exercise level. With the establishment of the most pronounced period of negative phase a slight but steady increase occurred in the milligrams of albumin present per 100 cc. of urine. The absolute amount of albumin continued to rise in the urine secreted during three successive 20 minute intervals following the cessation of blood pressure readings. There seem to be two albuminurias occurring, one during the exercise and the other during the post-exercise period of low pulse pressure.

With the use of the electrodynamic brake bicycle ergometer, various bouts of exercise were studied, to observe especially the influence of speed of working upon the incidence of albuminuria. It failed to occur after the types of exercise recorded in table 1. The exercises were all carried on at a slow or moderate pedalling rate. After the two long rides cardiovascular stabilization occurred rapidly and no lasting negative phase appeared. The short rides were very severe because the field currents were

TABLE 1

Showing the speed, duration and severity of various bouts of work which failed to produce albuminuria

TRIAL	SUBJECT	PEDALLING RATE	FIELD CURRENT	LOAD TERMINAL VOLTAGE	DURATION OF RIDE	RATE OF WORKING
		r.p.m.	*amperes*		*minutes*	*kgm.m/min.*
1	B. L	48	8.0	3.0	5	547.8
2	B. L	52	6.0	3.0	5	547.8
3	F. A. H.	61	3.4	2.4	60	365.3
4	B. L.	64	7.0	4.0	2	976.91
5	F. A. H.	68	6.0	4.0	5	976.91
6	E. L.	70	4.0	3.0	30	547.8
7	E. L.	75	5.0	4.0	5	976.91

relatively high. Post-exercise urine was collected approximately twenty minutes after these rides to allow sufficient time for the collection of an adequate sample. During this brief post-exercise period there were as yet no evidences of a deep negative phase, the reductions in the amplitude of the pulse pressure being irregular and evanescent.

In 1911 Lowsley had demonstrated on seven cases that a relationship exists between the post-exercise fall in pulse pressure and albuminuria following exercise, and had found that the quantity of protein appearing in the urine is greater the more extensive the period of low pulse pressure. He noted that short, rapid and exhaustive exercise tended to produce a marked and prolonged negative phase. With these considerations in mind, short rapid and severe bouts of exercise were instituted and the amount of albumin so induced was quantitatively determined.

The subjects of the experimentation were young women between the

ages of eighteen and thirty, either university students or faculty members. The group as a whole was above the average in neuro-muscular development, all having had professional training in physical education. They were familiar with physiological experimental technique and had all ridden the bicycle ergometer at some time prior to this research.

Immediately upon coming to the laboratory the subjects collected urine sample 1 and then sat on the bicycle until cardiovascular stabilization had occurred. The field current of the electrodynamic brake ergometer was set to give the desired load and the subject kept her speed of pedalling constant by maintaining the voltmeter needle on a given point. The revolutions per minute were estimated from the readings of a counter actuated by an eccentric on the armature shaft. Since not enough urine

TABLE 2

Showing the relationship of post-exercise albuminuria to the depth and duration of the negative phase within twenty minutes after a series of bicycle ergometer rides of the following speed, duration and severity

Field current—3.2 amperes. Load terminal voltage—3.5 volts. Pedalling rate—98 R.P.M. Duration of ride—5 minutes. Rate of working—762.36 kgm.m/min. Total work done—3,811.8 kgm. m.

	RIDE									
	1	2	3	4	5	6	7	8	9	10
Negative phase in pulse pressure during first 20 minutes post-exercise:										
Duration in minutes	0	0	0	1	3	5	6	7	9	10
Maximal depth in mm. Hg	0	0	0	2	4	10	17	8	7	8
Albumin in mgm./100 cc. of urine, 20 minutes after cessation of the exercise	48	25	47	55	49	35	30	10	8	25

accumulated during the short bouts of work to permit the collection of an adequate sample immediately after its cessation, the subject remained seated on the wheel and the blood pressure was observed at 60 second intervals for twenty minutes after the ride. Urine sample 2 was then taken. All urine samples were centrifuged and tested quantitatively for albumin, using the sulphosalicylic acid turbidimetric method (Kingsbury, Clark, Williams and Post, 1926).

Subject E. L. did a short, rapid bout of exercise on ten different days. Table 2 is a record of her pulse pressure findings during the immediate post-exercise period, and of the quantity of albumin present in the urine twenty minutes after the termination of the exercise.

All but one of the pre-exercise urine samples showed albumin in traces,

never exceeding 6 mgm./100 cc. Because of the persistence of these, complete kidney function studies were made. The blood NPN was 32 mgm./100 cc., very slightly above the usual limits of normal, but the PSP, urea concentration and Mosenthal tests conformed to standard. The negative phase in pulse pressure appeared after seven of the rides but every post-exercise urine sample contained relatively large quantities of albumin. It is of especial interest to note that following the three rides which failed to produce a post-exercise subnormal fall in the amplitude of the pulse pressure during the first twenty minutes after the cessation of exercise, an average of 40 mgm. of albumin was contained per 100 cc. of urine, in comparison with an average of 30 mgm./100 cc. in the urine samples collected after the seven rides which did induce an immediate negative phase.

TABLE 3

Showing the relationship of post-exercise albuminuria to the depth and duration of the negative phase within twenty minutes after a series of bicycle ergometer rides of the following speed, duration and severity, carried on by ten different subjects

Field current—3.2 amperes. Load terminal voltage—3.4 volts. Pedalling rate—averaged 92 R.P.M. Duration of ride—3 to 5 minutes. Rate of working—697.88 kgm.m/min.

	SUBJECT									
	DK	JK	MK	MB	DT	MM	DC	FH	KT	RP
Negative phase in pulse pressure during first 20 minutes post-exercise:										
Duration in minutes	2	4	9	11	11	12	12	13	13	13
Maximal depth in mm. Hg	2	6	10	12	12	26	28	12	12	16
Albumin in mgm./100 cc. of urine, 20 minutes after cessation of the exercise	95	95	15	90	85	40	10	35	3	3

The average albumin in the urine which was collected after the three rides which showed the most prolonged negative phase equalled only 14.3 mgm./100 cc., being inversely related to the pulse pressure phenomenon.

A series of different subjects rode the ergometer with load and speed conditions similar to those which had invariably induced post-exercise albuminuria in E. L. The average subject found the load light, but the rate of pedalling was too high to be maintained steadily without great effort. Table 3 gives the essential findings.

The bout of work was within the functional ability of six of the subjects. One completed the ride but had great difficulty doing so because of incoordination at so high a pedalling rate. Two subjects found the muscular exertion so severe that they were forced to stop at the end of three minutes,

one complaining of palpitation, the other of dyspnea. Four minutes of pedalling at these speed and load conditions once brought on perioral blanching, then extreme pallor, severe dyspnea with shallow sighing respirations and a feeble irregular pulse. From the above evidences we may conclude that the bout of work was approximately as severe as could be tolerated by the particular group acting as subjects.

Post-exercise albuminuria occurred in every case, the amount of albumin averaging 47 mgm./100 cc. of urine. A negative phase also appeared in every case, on the average, ten minutes after the cessation of the ride. The mean negative phase of the group as a whole was relatively prolonged and deep. There was, however, no definite relationship between the appearance of albuminuria and the magnitude of the immediate post-exercise reduction in the amplitude of the pulse pressure to a level below the pre-exercise normal.

TABLE 4

Showing the amount of albumin present in urine samples collected 20 minutes after bouts of equivalent work performed at high and low speeds

Load terminal voltage—3.4. Kgm.m/min.—697.88. Duration of ride—5 minutes

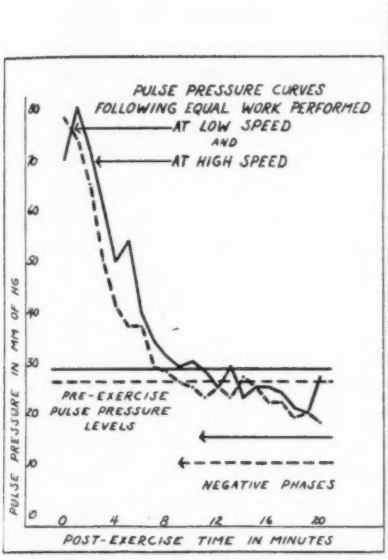
FIELD CURRENT	PEDALLING RATE	ALBUMIN IN URINE					
		DT	MM	JK	EL	MB	Average
amperes	*r.p.m.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	
3.2	94	85	40	95	55	90	73
5.2	65	0	2	6	10	4	4.4

Five subjects of the above series, who had shown relatively large amounts of albumin after the bout of exercise carried on for a short time at high speed, did an equivalent amount of work in an identical period of time with a heavy load at low speed. Table 4 contains the albumin findings.

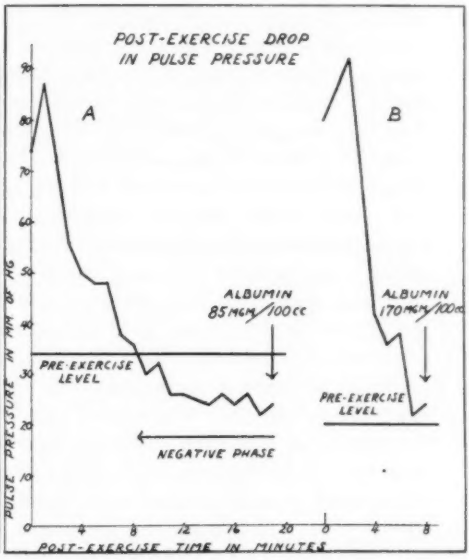
It is to be noted that a simple change in the field current was the only manipulation necessary for the establishment of the desired load and speed conditions. As long as the voltmeter needle was held at the point maintained during the fast ride, the energy input remained constant, the pedalling rate necessary to keep the needle at this point being automatically reduced with the increase in field current.

The subjects all showed relatively large amounts of albumin in the urine after the rapid piece of exercise, and the same piece of work done at low speed was followed by very small amounts. The depth and duration of the reduction in the amplitude of the pulse pressure was approximately the same after both bouts of exercise, although the albumin findings varied so markedly. Graph 4 clearly demonstrates the lack of an etiological relationship between the albuminuria and the pulse pressure under the speed and load conditions studied.

Graph 5 presents one more evidence of a lack of causal relationship between the albuminuria following short, violent exercise of speed and the post-exercise fall in pulse pressure. Subject D. T. had no albumin in the pre-exercise urine samples, but showed consistent albuminuria following various types of exercise, both experimental bouts of work and sport participation. She was able to collect adequate urine samples at relatively short intervals of time. Eight minutes after the cessation of work and



Graph 4



Graph 5

Graph 4. Showing the lack of relationship between albuminuria and the pulse pressure after equivalent bouts of work.

Graph 5. Showing the absence of an etiological relationship between the post-exercise negative phase and albuminuria. Rides A and B were identical. One urine sample was collected during the period of low pulse pressure, the other before the onset of the negative phase. Both contained albumin.

before the onset of the negative phase in pulse pressure, an abundance of albumin appeared in the urine.

Discussion. We have seen that the duration of a piece of exercise and the absolute external work done may remain constant while the speed varies. Under such conditions more energy is required to perform the rapid work than the slow because of the greatly increased internal frictional loss (Hill, 1927). The amount of oxygen required during exercise at high

speed is greater than that at low and when the response of the cardiovascular and respiratory systems is such that the intake exactly balances the requirement, the subject is in the steady state and lactic acid remains at a constant value. As the speed of work is increased and the oxygen requirement goes up, lactic acid accumulates in the blood. (Hill and Lupton, 1923.) Fletcher and Hopkins (1907) had demonstrated that lactic acid production in isolated muscle is due to oxygen want. Feldman and Leonard Hill (1911) came to the same conclusion after a study of the effect of oxygen inhalation upon the production of post-exercise urinary lactic acid in man. The lactic acid in the blood at rest is equal to 10 to 20 mgm./100 cc. (Long, 1924). After severe exercise it increases. In 1909 Ryffel found that the post-exercise excess of lactic acid after a short violent bout of work does not disappear from the urine until 30 minutes after the cessation of the exercise, a still longer period of time being necessary for the return of the blood lactic acid level to normal. In 1923 Barr, Himwich and Green found marked changes in the acid-base equilibrium of the blood after short periods of heavy muscular exercise; the blood became less alkaline, the arterial CO_2 was reduced, the CO_2-combining capacity diminished, and lactic acid concentration increased. They believed these changes to be dependent upon the relative rates at which lactic acid is produced and removed. The CO_2-combining capacity of the blood only gradually returned to normal and recovery was not yet complete 50 minutes after the termination of muscular exertion. Ryffel (1909) observed that the acidity of the urine definitely increased after short violent bouts of exercise. In his experimental studies on nephritis, Fischer (1912) induced albuminuria in rabbits by the continuous intravenous injection of hydrochloric acid. The urine also contained red blood corpuscles, epithelial cells and casts. Post and Thomas (1923), studying another non-nephritic albuminuria, found that every case of orthostatic albuminuria, with rare exceptions, could be made albumin-free by neutralization or mild alkalization of the urine, an etiological relationship existing between the acidity of the urine and the permeability of the renal tissue.

SUMMARY

1. Two types of post-exercise albuminuria exist. In addition to that occurring a relatively long time after the cessation of moderate, prolonged and steady exercise, another appears during long bouts of rapid and exhausting work or shortly after the termination of brief, violent exercises of speed.

2. The albuminuria occurring during exercise or shortly after its cessation is unrelated to the concomitant variations in pulse pressure, but bears an etiological relationship to the speed of doing work, occurring only after violent and rapid muscular exertion.

3. The findings may be explained by the hypothesis that exercise of speed brings about a generalized systemic increase in acidity which alters the permeability of the renal tissue to blood proteins, in consequence of which albumin appears in the urine.

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HEMODYNAMICS OF ARTERIOSCLEROSIS

Influence of Change of Coefficient of Volume Elasticity on Circulation

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In a previous publication (1) we have shown that the total energy consumption of the heart decreases slightly when the coefficient of volume elasticity of the large artery system is increased many times (10) provided that the change in coefficient of volume elasticity is not accompanied by a change in lumen of the large artery system. It was also shown that the external work of the left ventricle is not increased, that the diastolic blood pressure falls more than the systolic rises, that the coronary flow is slightly decreased, and that the volume flow in the systemic system does not decrease when the rigidity of the arterial system is increased to many times the normal.

In our previous work we brought about a decrease in the coefficient of volume elasticity by introducing air chambers along the large arterial system made of glass. The "effective" bore of the system remained the same when the large artery system was connected to the air chambers. In consequence of this there was no difference in the arterial resistance in the two conditions of rigidity. In arteriosclerosis of the large artery system there are static and dynamic changes of bore which must be considered. Static changes are widening of the lumen due to passive stretching of the walls and narrowing of the lumen due to intima thickening. Dynamic change of lumen is due to increase in diameter consequent upon increase of blood pressure within the tube in going from diastolic to sytstolic pressure during each heart cycle. The static changes can be easily evaluated. Any increase in bore reduces the resistance to blood flow approximately as the inverse ratios of the fourth powers of the diameters.[1] A decrease in bore increases the resistance to blood flow as the fourth power of the decrease in diameter of the lumen of the vessel.

[1] Poisseuille's law does not hold exactly but is a fair approximation and describes the effect of lumen change on resistance to flow well enough for the purpose of this discussion.

A rough calculation will show that the dynamic change in lumen is probably of little consequence to blood flow in the large arterial system. Let us assume that we have two tubes of the same diameter at 80 mm. Hg internal pressure. The one tube is of glass and therefore does not enlarge when the internal pressure is raised. The other tube has the same coefficient of volume elasticity as the human large arterial system. The resistance of this tube to blood flow must decrease approximately 14 per cent when the blood pressure within the tube increases from the normal diastolic to the normal systolic pressure because A. V. Hill (2) has shown in his determinations of the coefficient of volume elasticity of the adult human large artery system that the volume of the large arteries increases approximately 6.6 per cent when the pressure within is increased from 80 mm. Hg to 120 mm. Hg.[2] But the diameter of the blood vessel is not continuously that of systolic pressure. The vessel widens from its diastolic diameter to its systolic and then back to the diastolic diameter during each pulse wave. Using the best optic manometer curves of aortic pressure for calculation, it can be shown that the mathematical "mean" increase in diameter of the vessel lumen is less than 70 per cent of the maximum systolic increase in diameter. In other words, the widening of the large arterial system during a complete heart cycle reduced the "effective" resistance of the system not more than 10 per cent. If we assume that the resistance of the large arterial system is 25 per cent of the whole vascular resistance, then we see that the total reduction in resistance to blood flow due to widening of the lumen of the large artery system during systole is only about 2.5 per cent of the total resistance; an almost negligible decrease in resistance.

Despite the results of the foregoing calculation we know that it would be impossible to convince many physiologists and clinicians that a great increase in rigidity of the large artery system probably would not increase the work of the left ventricle unless the proof was experimentally demonstrated. The idea of E. A. Weber (3) that the work of the heart is greatly reduced by the substitution of elastic tubes for rigid tubes is firmly fixed in the minds of most physiologists and clinicians. In order to prove experimentally that the work of the heart is not increased appreciably by a great increase in rigidity of the large arteries and in order to study other factors of the circulation when the coefficient of volume elasticity is increased, the following experimental method was devised. See figure 1.

The heart-lung preparation of Starling was used in connection with an artificial large artery system whose coefficient of volume elasticity could be changed at will from values about half that of the normal adult human large artery system to values nearly ten times that of the normal adult human large artery system. This large artery system consisted of a piece

[2] This calculation of decreased resistance from increase in lumen is based upon Poisseuille's equation and is only approximate.

of thin walled rubber tubing of diameter about 12 mm. and length 100 cm. kindly constructed by the research department of the Goodrich Rubber Company[3] so as to have a coefficient of volume elasticity about half that of the normal adult large artery system. This tube was enclosed within a heat insulated lead pipe of internal diameter 35 cm. The lead pipe had five stopcocks inserted at equal intervals along its wall. These stopcocks were connected to glass jars by rubber tubing. The rubber tubing within the lead pipe was filled with physiological salt solution under a pressure of about 25 cm. H_2O and clamped off. Then water was filled into the lead pipe, care being taken to exclude all air bubbles. The stopcocks were then closed and the rubber tube connected at one end to the aortic cannula of the heart-lung preparation and at the other end to the artificial resistance of the system and blood allowed to flow from the heart through the rubber tube, and artificial resistance into the venous reservoir of the heart-lung preparation. With the stopcocks closed the coefficient of volume elasticity of the large artery tube is that of the water mantle, i.e., extremely high rigidity; when the stopcocks are opened to the air the coefficient of volume elasticity of the tube is that of the rubber tubing or approximately half the normal. Coefficients of any desired value between half that of the normal artery and that of water could be obtained when the stopcocks were connected to bottles of various capacity filled with air at atmospheric pressure and with the cocks open. Thus it is possible to quickly vary the rigidity of the artery from a value half that of the normal large artery system of the adult to any value desired up to that of an almost incompressible fluid by simply closing and opening five stopcocks in the lead jacket. In carrying out the experiments the stopcocks were closed as nearly as possible at the end of diastole in order that the diameter of the tubing in the state of increased rigidity would be as nearly as possible that of the diastolic condition in the less rigid state. That we succeeded in this is shown by the fact that we could repeat the process of closing and opening stopcocks many times with nearly perfect repetition of the volume curves of the ventricles and nearly perfect repetition of the blood pressure curves! The large artery system is connected to the arch of the aorta by a cannula of 9 mm. internal bore inserted into the descending aorta up close to the aortic valves. At the other end it is connected by a glass tube 10 cm. long and bore 3.5 mm. with an artificial resistance for regulating blood pressure to any desired value. The artificial resistance is connected to the venous reservoir as in figure 1. The water mantle around the large artery is filled with water at about 45°C. This cools to a temperature such as to keep the blood entering the superior vena cava fairly constantly at about 32 to 35°C. after being put into the lead jacket. The resistance of the large artery

[3] We wish to thank Dr. J. W. Schade for his kindness in having tubing constructed according to our specifications by the Goodrich Rubber Company.

system in our setup at the pressures we worked with was 10 to 12 per cent of the whole vascular resistance. A mercury manometer was connected to the aortic cannula for registration of blood pressure. In a few instances a Wiggers optical manometer was used to get more accurate blood pressure curves. A Henderson cardiometer was inserted over the heart to measure the volume of the ventricles. This cardiometer was connected by copper tubing to a Wiggers segment capsule for recording the changes in volume

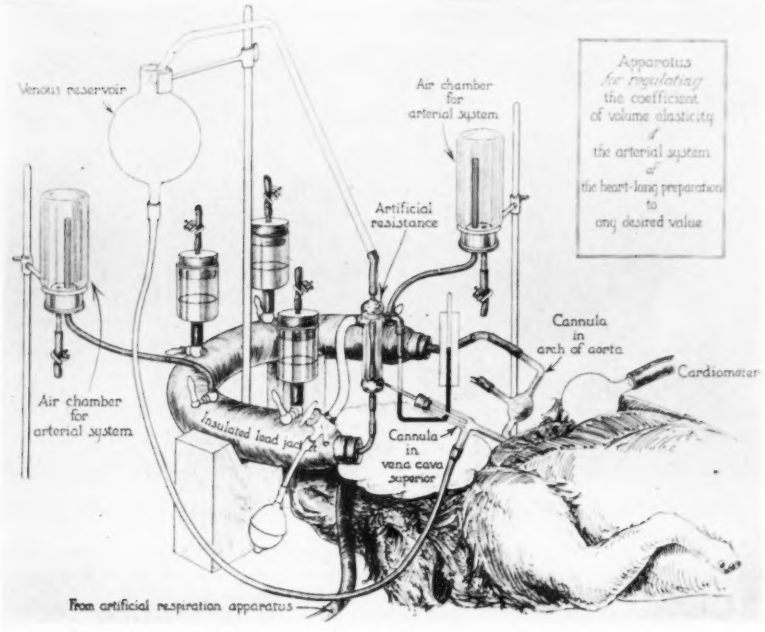

Fig. 1. Apparatus for studying effects of arteriosclerosis upon the circulation in the heart-lung preparation. In all our experiments, the bottles connected to the stopcocks were kept open to the atmosphere so that with the stopcocks opened, coefficient of volume elasticity of the rubber tubing within the lead pipe and water mantle is that of the rubber tubing.

of the ventricles. The cardiometer tracings were calibrated by injecting 20 cc. of air into the system through a side tube and the corresponding descent of the lever marked on the drum. Time was measured in seconds on the drum by a Jacquet chronometer. The lower position of the chronometer line was adjusted to the 0 point of the mercury manometer and is therefore the base line of the mercury manometer. A signal marked the moment of closing or opening the stopcocks on the lead jacket, or in other

words the time of transition from arteriosclerosis to normal artery and vice versa.

The circulating minute volume, i.e., the true minute volume minus the coronary flow, was measured by means of a stopwatch graduated to 0.1 second and a measuring cylinder marked off for volumes of 150 cc. Blood

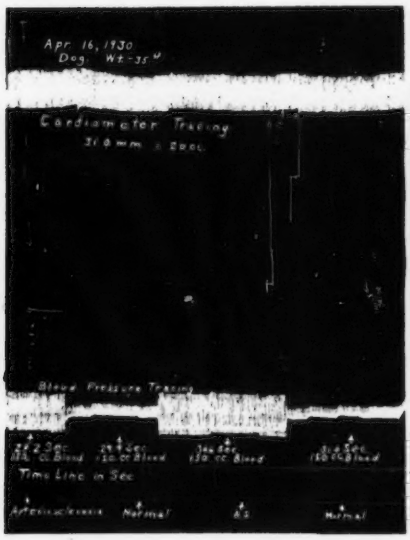

Fig. 2

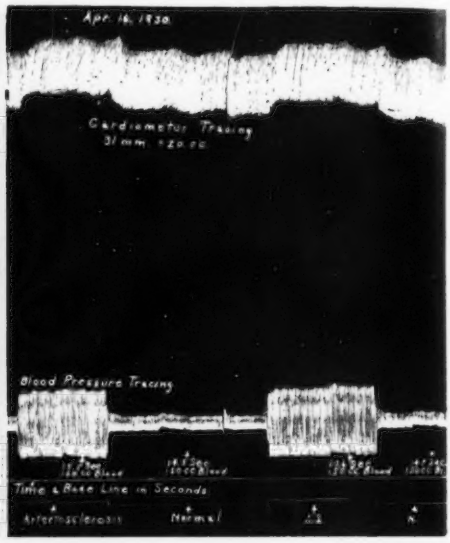

Fig. 3

Fig. 2. Tracing of experiment of April 16, 1930. Upper tracing cardiometer volume; lower tracing mercury manometer. Moment at which out-flow from venous end was measured indicated by arrows below blood pressure tracing. Time of out-flow of 150 cc. of blood marked in seconds. Upstroke of cardiometer tracing is systole; downstroke diastole. During the arteriosclerosis the diastolic volume of the heart is approximately 1.5 cc. smaller than during the normal. Pulse pressure in arteriosclerosis is approximately three times as great as in the normal.

Fig. 3. Experiment of April 16, 1930, after increasing the artificial resistance very slightly and increasing the inflow from the venous reservoir into vena cava superior. Pulse pressure in arteriosclerosis approximately four and one-half times as great as in normal. During arteriosclerosis, diastolic volume of heart is approximately 3 cc. smaller than in normal.

leaving the end of the rubber tubing and entering the venous reservoir could be diverted at will into the cylinder. The time taken to fill the 150 cc. was determined by the stopwatch. The minute flow could be measured in this way with an accuracy of $\pm$ 3 per cent.

The cardiometer was always carefully placed around the ventricles and

care taken to avoid leaks. It was impossible for us to determine the accuracy of our measurements of stroke volume from the cardiometer tracings but we believe the error was not large. The error in stroke volume determines the error in the minute volume determination because the minute volume is stroke volume times the number of heart beats per minute. The latter can be accurately determined. Coronary flow was determined

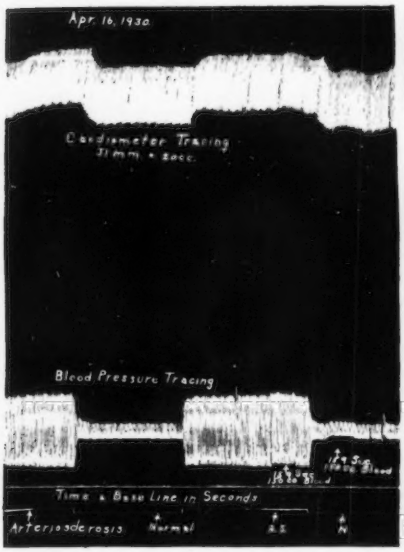

Fig. 4

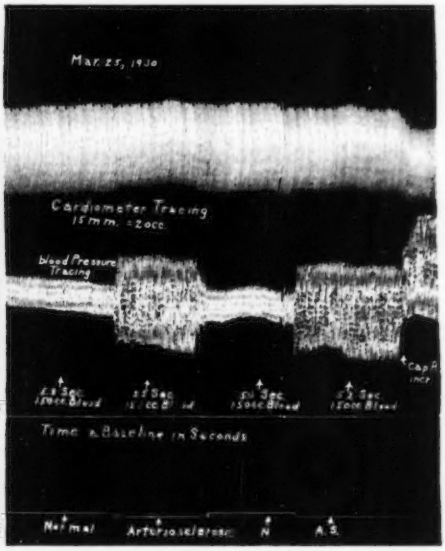

Fig. 5

Fig. 4. Experiment of April 16, 1930. Same as figure 3 excepting that tracing was taken ten minutes later. Pulse pressure is approximately four times as great in arteriosclerosis. During arteriosclerosis, diastolic volume of the heart is approximately 3.5 cm. less than in normal.

Fig. 5. Experiment of March 15, 1930. In this experiment in which the minute volume is very large and the blood pressure moderately elevated, we see that the diastolic volume of the heart is approximately 3 cc. smaller in arteriosclerosis than in the normal. Pulse pressure in arteriosclerosis is approximately 2.7 times as large as in the normal and the circulating volume approximately the same in both conditions.

by subtracting the relatively accurate circulating volume from the minute volume of the left ventricle as determined from half the cardiometer stroke volume times the heart beat. The absolute error in this determination is the error in the cardiometer stroke volume. We believe that the minute volume determination is accurate to 10 per cent. The

diastolic position of the cardiometer tracing would sometimes stay constant for a half-hour or longer provided there was no change made in the inflow into the superior vena cava and no change made in the artificial resistance and provided the temperature remained constant. On the other hand the coronary flow which is determined by a difference in quantities many times larger is not nearly as accurate (in percentage) as the stroke volume.

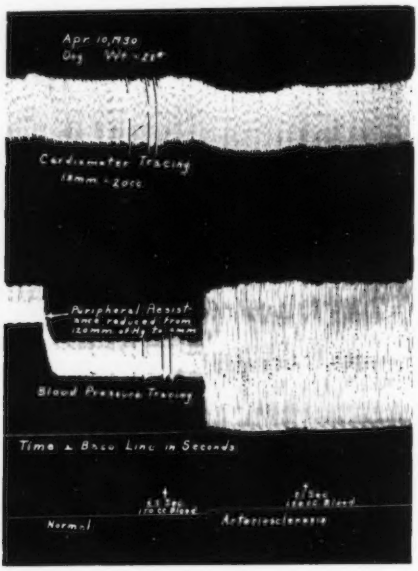

Fig. 6. Experiment of April 10, 1930. Working with a very low blood pressure and a very high stroke output, we see that during arteriosclerosis there is a dilatation of the heart of approximately 2 cc. The flow through the coronaries with the stroke volume is very much less during arteriosclerosis; at the same time the flow through the rest of the arterial system is the same as in the normal, the result being a very much reduced flow through the coronary arteries with an approach to the hypodynamic condition.

In determining the coefficient of volume elasticity a clamp was placed on the rubber tubing connecting the artificial resistance to the glass tube leading to the venous reservoir, thus closing the system at the venous end of the artificial resistance. The whole system was kept filled with blood and a record syringe filled with saline was connected to the aortic end of the aortic cannula; 2 cc. amounts of saline were injected into the system with the stopcocks of the lead jacket closed (arteriosclerosis) and the

corresponding rises in pressure recorded on the drum by the mercury manometer. With the stopcocks of the lead jacket open (normal artery) 20 cc. amounts of saline were injected into the aortic cannula and the corresponding rises in pressure recorded on the drum by the mercury manometer. We have expressed rigidity or coefficient of volume elasticity in terms of 1 per cent increase in volume to corresponding rise in millimeters mercury pressure. It will be seen that what we determined by this calibration is the coefficient of volume elasticity of the whole system from the heart to the venous end of the artificial resistance with the mercury manometer in the system. This value is much less than the coefficient of volume elasticity of the tube within the lead jacket when the stopcocks are closed because the artificial resistance adds a tube of very low rigidity and the mercury manometer itself is a tube of even lower rigidity. When the stopcocks are opened (normal) the elastic effects of the artificial resistance and the mercury manometer make very little change because they both have low coefficients of elasticity of the same order of magnitude as the rubber tubing in the jacket and moreover, their volume is very small in comparison to the volume of the tubing within the lead jacket. In determining the volume of the system, all the fluid in the system including that in the artificial resistance, that within the tubing in the jacket, the content of the arterial cannula and the fluid in the connections of the mercury manometer were measured. It can be said that the values determined therefore are very much lower than the value of the coefficient of volume elasticity for the large artery system alone in the condition of great rigidity or arteriosclerosis and are just a little high for the value of the coefficient of volume elasticity of the large artery tubing in the normal condition. The error in our experimentally determined coefficients of volume elasticity due to the fact that the tip of the aortic cannula reached only to within 1.5 or 2 cm. of the root of the aorta is of such an order of magnitude that it can be neglected.

We shall consider that the diastolic volume of the heart is an index of the total energy consumption of the heart as first proved by Starling and Visscher (4) and that as a first approximation the product of minute volume and mean blood pressure is equal to the external work of the left ventricle. The energy consumption of the heart increases if the heart dilates and decreases if the heart gets smaller, provided the inherent contractile property of the heart muscle remains the same. Everything else being equal, the external work of the left ventricle may be looked upon as being increased if the diastolic volume of the heart increases and it may be looked upon as being decreased if the diastolic volume of the heart decreases. Unchanged diastolic volume means unchanged amount of external work performed by the heart.

Experiment of April 16, 1930. Calibration of elastic coefficient in terms of percentage increase of arterial system to millimeter of mercury increase in pressure or $\frac{\text{mm. Hg increase in pressure}}{\% \text{ increase in volume}}$ = "effective" elastic coefficient or rigidity of arterial system.

Stopcocks closed (arteriosclerosis)

2 cc. fluid injected into system of 240 cc. capacity gives a rise of 48 mm. Hg in pressure within this system.

$\frac{2}{240}$ = 0.83 per cent increase in volume for 48 mm. Hg.

One per cent increase in volume for 58 mm. Hg.

A. V. Hill's normal artery between 80–120 mm. Hg showed 1 per cent increase in volume for 6 mm. Hg.

In this arterial system with stopcocks closed there is a rigidity 9.5 × the "mean" normal artery of A. V. Hill at normal pressure; i.e., an extremely severe arteriosclerosis.

Stopcocks open (normal)

20 cc. fluid injected into arterial system gives a rise of 28 mm. Hg.

$\frac{20}{230}$ = 8.7 per cent increase in volume for 28 mm. Hg pressure.

1 per cent increase in volume for 3.2 mm. Hg.

This rigidity is approximately ½ that of A. V. Hill's "mean" normal artery between the pressures of 80–120 mm. Hg.

Rigidity with stopcocks closed is 18 times rigidity with stopcocks open.

Stopcocks open (Normal)				*Stopcocks closed (arteriosclerosis)*	
Sys. B.P.	56	Mean B.P.		66	Mean B.P.
Dias. B.P.	43	49.5		28	47
Stroke Vol.	5.6	cc.	Figure 2.	5.2	cc.
Minute Vol.	504	cc.	Heart rate 90	468	cc.
Circul. Vol.	299	cc.		294	cc.
Coronary flow.	205	cc.		174	cc.

Inflow increased. Artificial resistance slightly increased.

Sys. B.P.	58	Mean B.P.		81	Mean B.P.
Dias. B.P.	44	51		19	50
Stroke Vol.	9.0	cc.	Figure 3.	8.7	cc.
Minute Bol.	810	cc.	Heart rate 90	783	cc.
Circul. Vol.	615	cc.		615	cc.
Coronary flow.	195	cc.		168	cc.

ten minutes later

Sys. B.P.	58	Mean B.P.		80	Mean B.P.
Dias. B.P.	43	50.5		18	49
Stroke Vol.	8.4	cc.	Figure 4.	8.0	cc.
Minute Vol.	756	cc.	Heart rate 90	720	cc.
Circul. Vol.	565	cc.		565	cc.
Coronary flow.	191	cc.		155	cc.

Stroke Vol. = Output of one ventricle. Obtained by multiplying the height of cardiometer curve in millimeters by 20 and dividing by 2 times the increase in height of cardiometer tracing when 20 cc. are forced into the cardiometer system from a record syringe connected to the system.

Minute Vol. = Total output of left ventricle calculated from stroke volume multiplied by heart rate.

Circul. Vol. = Volume of flow as measured with stopwatch and calibrated measuring cylinder on blood flowing out of venous system into venous reservoir.

With stopcocks closed, i.e., in arteriosclerosis, the diastolic volume is less, showing that the energy consumption of the heart is less in arteriosclerosis. This is due to the decrease in external work of the left ventricle largely. The arithmetical mean pressure falls slightly and the minute

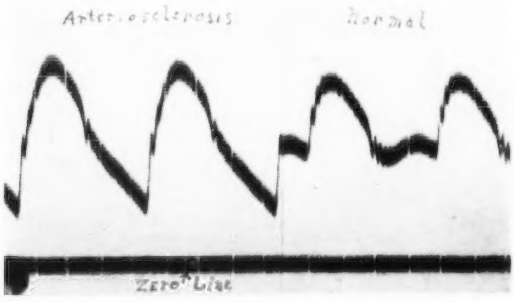

Fig. 7. Optical records of blood pressure in the aorta. First two curves are arteriosclerosis, systolic blood pressure being 97 and diastolic blood pressure 28. The last two curves are taken from the normal artery, systolic blood pressure is 86 and diastolic blood pressure 56. The aortic pressure curves taken with optical manometers on other days did not always show the secondary wave in diastole, but the fall of pressure during diastole was always very slight in the normal because of the low coefficient of elasticity of the artery.

volume also. The external work of the heart measured by their product is definitely less. In fact blood pressure curves taken with the optical manometer show an even greater reduction in diastolic and arithmetical mean pressures (see fig. 7). The coronary flow is always definitely less in arteriosclerosis.

Experiment of March 25, 1930. Calibration of elastic coefficient in terms of precentage increase in volume of arterial system to millimeter of mercury increase in pressure.

Stopcocks closed (arteriosclerosis)

2 cc. fluid injected into system of 260 cc. capacity gave a rise of 40 mm. Hg in pressure within this system.

1 per cent increase in volume for 52 mm. Hg or a rigidity 8.6 × the "mean" normal artery of A. V. Hill.

Stopcocks open (normal)

20 cc. fluid injected into system of 290 cc. capacity gave a rise of 20 mm. Hg in pressure within this system.

1 per cent increase in volume for 2.9 mm. Hg or a rigidity ½ the "mean" normal artery of A. V Hill.

The rigidity with stopcocks closed is 18 times the rigidity with stopcocks open.

Stopcocks open (Normal)				*Stopcocks closed (arteriosclerosis)*	
Sys. B.P.....	120	Mean B.P.		140	Mean B.P.
Dias. B.P.....	90	105		58	99
Stroke Vol.....	26.0	cc.	Figure 5.	24.5	cc.
Minute Vol.....	2028	cc.	Heart rate 78	1911	cc.
Circul. Vol.....	1665	cc.		1635	cc.
Coronary flow.....	363	cc.		276	cc.

With the minute volume very large we see that the diastolic volume of the heart decreases in arteriosclerosis corresponding to a decrease in energy consumption of the heart. The external work of the heart also decreases as measured by the product of minute volume and arithmetical mean pressure.

Experiment of April 10, 1930.

Stopcocks closed (arteriosclerosis)

2 cc. fluid injected into system of 200 cc. capacity gives 40 mm. of mercury increase within this system.

1 per cent increase in volume for 40 mm. Hg or a rigidity 6.6 times the rigidity of A. V. Hill's normal arterial system.

Stopcocks open (normal)

20 cc. fluid injected into system of 240 cc. capacity gives 24 mm. Hg rise in pressure within this system.

1 per cent increase in volume for 2.8 mm. Hg or a rigidity ½ that of A. V. Hill's normal.

The rigidity with stopcocks closed is 14 times that with stopcocks open.

Stopcocks open (Normal)				*Stopcocks closed (arteriosclerosis)*	
Sys. B.P.....	82	Mean B.P.		124	Mean B.P.
Dias. B.P....	50	66		0	62
Stroke Vol...	1611	cc.	Figure 6.	15.2	cc.
Minute Vol..	1207	cc.	Rate of heart 75	1140	cc.
Circul. Vol...	1084	cc.		1110	cc.
Coronary flow	123	cc.		30	cc.

In this experiment we see a dilatation of the heart of 4 cc. in the condition of arteriosclerosis. This is the only experiment we have showing a dilatation of the heart in arteriosclerosis. The cause for this is to be found in the inadequate coronary flow during the arteriosclerotic period of the experiment. Though the external work of the heart is decreased in arteriosclerosis, yet the heart dilates because it is

becoming hypodynamic due to inadequate coronary blood flow. The work of the left ventricle in this heart is 0.94 kilogram-meter per minute and reckoning the work of the right ventricle at ⅓ of this, the external work of the heart is 1.13 gram meters per minute or 0.028 calorie which is equivalent to 5.8 cc. of oxygen consumption. If we assume the mechanical efficiency to be 40 per cent, then the total oxygen consumed by heart to perform this work will be 14.5 cc. Thirty cubic centimeters of arterial blood only contains 6 cc. of oxygen. Therefore there is insufficient oxygen supply for the work of the heart muscle assuming that the coronary flow as determined above is correct.

In figure 7 we show aortic pressure curves recorded by a Wiggers optical manometer at 10 feet distance. These curves are very accurate records of the blood pressure relations in arteriosclerosis and in the normal. The first two aortic pressure curves were taken with stopcocks closed (arteriosclerosis) and a coefficient of volume elasticity eight times that of the normal adult large artery system. The last two curves give the pressure relations in the aorta when the stopcocks were open (normal) and the coefficient of volume elasticity was half that of the normal adult large artery system. Calibration of the optical manometers show

Arteriosclerosis			*Normal*	
Sys. B.P.	97	Mean B.P.	86	Mean B.P.
Dias. B.P.	28	62	56	71

By integration of the pressure curve in arteriosclerosis and the pressure curve in the normal artery and dividing by the abscissa, we have determined the "mathematical" mean pressures in the two conditions.

	"Mathematical" mean pressure for one whole cycle
Arteriosclerosis	62
Normal	66

Blood is thrown into the aorta only from the beginning of the systolic rise to the diastolic notch and therefore the mathematical mean pressure during this period times the minute volume is a better index of the work of the left ventricle than the minute volume times the mathematical mean pressure for a whole pulse cycle. By integration we have determined this as approximately the same in both conditions.

	Mathematical mean pressure for systole
Arteriosclerosis	79
Normal	78

If we wish to get an idea of the magnitude of the blood pressure effective in causing flow through the coronary arteries, it is more nearly correct to integrate the pressure curves from the diastolic notch to the end of diastole for most of the flow in the coronary arteries occurs during diastole. We have done this for the above curves and find for the true mean pressure during diastole:

Arteriosclerosis	41
Normal	52

The minute volume through the coronary arteries should be proportional to these figures. As a matter of fact the coronary flow in arteriosclerosis and in the normal, in our experiments, is very closely proportional to these figures, excepting the experiments of April 10th when the coronary flow during arteriosclerosis was only about 25 per cent of that in the normal system.

This investigation proves conclusively that a very great increase in the coefficient of volume elasticity of the large artery system does not increase the external work of the left ventricle nor increase the energy consumption of the heart; rather it leads to a small decrease in both these magnitudes. Moreover the minute volume in the arteries exclusive of the coronary arteries is not decreased in severe arteriosclerosis.

The only adverse factor that our investigation brought to light was a decreased coronary flow; in one instance the coronary flow was so small that the contractile property of the heart muscle decreased. The cause of this decreased flow is to be found in the lowered mathematical mean pressure during diastole. The systolic pressure rises in arteriosclerosis but the diastolic pressure decreases more than the systolic rises. More especially the mean pressure during diastole decreases greatly in the arteriosclerotic condition. The normal artery tends to hold up the pressure after the closure of the semilunar valves because the energy stored up in the stretched elastic walls tends to be transformed into pressure during diastole. This tendency to maintain blood pressure at a high level during diastole is very probably a very important consequence of the low coefficient of volume elasticity of the large artery system of man. The vagus effect upon the coronary arteries of the dog is destroyed in the heart-lung preparation so that we probably have a maximal bore in these vessels in this preparation as a rule. If the bore should be reduced let us say by intima thickening as in coronary arteriosclerosis or by any other mechanism, then with an increase in rigidity in the large arteries, the flow through the coronaries might be lowered to a point where the muscle would lose its inherent contractile properties.

Some observers (5), (6) have claimed that the flow through a tube of small coefficient of volume elasticity is very much greater than the flow through a tube of greater rigidity or that the flow through an organ is greater when using a pulsating pressure source instead of a constant pressure source. These observers have used experimental methods which throw little light on the problems of hemodynamics when the rigidity of the large artery system alone is greatly increased. Romberg (5) compared the outflow from two pieces of tubing, one of glass and the other of very easily stretched rubber, of the same diameter under zero pressure and the

two pieces of tubing making up the whole resistance of the system, whereas the resistance of the large artery system is less than 25 per cent of the whole vascular resistance. If we should have used a glass tube of internal diameter 12 mm. and our rubber tube of diameter 12 mm. when no pressure was within the tube and applied a pulsating pressure varying from 120–80 mm. Hg the outflow from the rubber tube would have been 90 per cent greater than from the glass tube because the rubber tube would have dilated in going from 0–80 mm. Hg and again it would periodically increase its bore in going from 80 mm. Hg to 120 mm. Hg. Fleisch (6) compared the outflow from whole organs as kidney when a pulsating pressure was acting, with the outflow produced by a constant pressure equal to the mean of the pulsating pressure. Here also the outflow was greater under pulsating pressure because the small arteries and veins dilated very much under increased pressure in systole because by Poisseuille's law there must be a greater outflow with pulsating pressure. On the other hand, we have only attempted to study the circulation as modified by a change in rigidity of the large artery system, the small artery, the capillary, and the venous systems remaining unchanged. Moreover we have attempted to keep the diastolic bore of the large artery system the same in both states, allowing the diameter to increase only during systole in the less rigid state. A more extensive review and discussion of other work in this field will be given when we publish our third paper on the subject taking up the problem of circulation when the rigidity of the small arteries and capillaries is altered.

CONCLUSIONS

Provided that the diastolic diameter of the rigid artery is the same as that of the less rigid artery and the resistance of the large artery system is about 10 to 15 per cent of the whole vascular resistance:

1. The energy consumption of the heart is not increased but is slightly decreased when the coefficient of volume elasticity of the large arteries is greatly increased.

2. The external work of the left ventricle is not increased when the coefficient of volume elasticity is greatly increased, rather the external work is slightly decreased in severe arteriosclerosis of the large arteries because the "mathematical mean" pressure during systole remains nearly the same and the minute volume decreases a little.

3. The flow through the coronary vessels decreases 20–25 per cent after severe increase in the rigidity of the large arteries because the "mathematical mean" pressure falls about this much during diastole, the period during which most of the flow takes place in the coronary arteries. Under certain circumstances, it may fall so far that the inherent contractile property of the heart muscle is impaired.

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THE PASSAGE OF UREA BETWEEN THE BLOOD AND THE LUMEN OF THE SMALL INTESTINE

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The purpose of this work was to determine whether or not urea will pass from the blood to the contents of the bowel, and to study the conditions of its passage. Twenty-one dogs were used. In some cases two and three series of experiments were run on each dog.

METHODS. Urea and ammonia determinations were made by the Folin-Wu distillation method in the earlier experiments. Later the aeration method was used. In our hands the aeration method proved more convenient and more constant in results. All final readings were made colorimetrically after Nesslerization. The urea content of the blood was found systematically higher by the Folin-Wu method which, it seems likely, is because in diluting to a definite volume before precipitation, the volume of protein precipitate is neglected. By this method the real dilution is less than the supposed, leading to high values. In the aeration method the blood was measured and treated with urease and aerated, allowing for no such error of dilution. The differences in the two methods are not great enough to interfere with their usefulness in this problem. The blank due to the urease, usually 1.7 mgm. per cent in routine determinations, and a correction due to caprylic alcohol passed over in the aeration process, were deducted in making calculations. Checks on known solutions showed these corrections to be valid.

An interesting source of error was encountered in these studies. When tap water was placed with Nessler's solution in any of various proportions there was no color change. It was therefore at first concluded that the city water contained none of the Nessler reacting substances. Later it was found that when Nessler's reagent reacted to ammonia (as in all the routine determinations) a small proportion (2 to 5 drops) of city water would cause a very serious clouding. A rather exhaustive study showed that the minerals in the city water actually caused the clouding, and that it would occur consistently, also that no clouding would occur unless at least a trace of ammonia was present. Rinsing in distilled water made glassware free from this effect, ordinarily, but it was found that occa-

sionally commercially distilled water also contained enough of the clouding substance to cause loss of unknowns. If the amount of clouding substance was very slight, then by rapidly making colorimetric determinations one might finish before the solution definitely clouded. This is hardly commendable. There was absolutely none of this trouble when a redistilled water was used for making dilutions and for rinsing.

All values for "urea nitrogen" unless otherwise specified include urea plus ammonia nitrogen.

Dogs were used under sodium amytal anesthesia. The dose was approximately 60 mgm. per kilo. In some of the work the amytal was injected into the thigh muscles. In the later experiments it was given intraperitoneally. Frequently the amytal anesthesia was supplemented by ether inhalation, usually for short intervals.

The bowel was cleaned in each dog by flushing with normal saline solution at body temperature. About four liters would be run through in slightly less than an hour in cleaning the bowel. The washings would be quite clear by this time. The clamping off effect of peristaltic waves blocked the free flow of the solution to some extent. However, a fluid pressure (elevation) of about 17 cm. generally proved sufficient to overcome such obstruction.

Solutions entered the small bowel through a rubber tube inserted into the middle third of the duodenum. Solutions left the small bowel through a larger rubber tube fastened into the ileum near the ileo-cecal junction. The bowel above and below this loop was tied off. Thus practically all of the small bowel was used as the loop for these tests. Samples were generally taken by inserting a Luer needle diagonally through the bowel wall each time. Generally about 8 cc. would be removed for determinations.

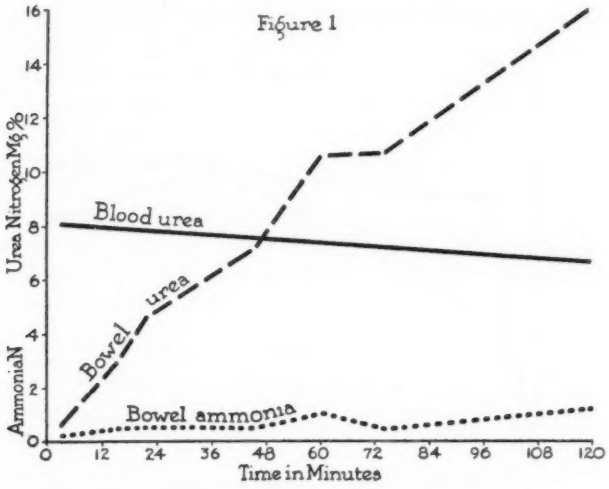
Results. When normal saline solution was placed in the small bowel, its urea content rapidly rose to that of the blood, as is shown for a typical experiment in figure 1. In many of the animals the bowel contents showed 6 mgm. per cent of urea nitrogen in five minutes. In fifteen minutes it reached 20 mgm. per cent. This was already slightly in excess of the blood urea nitrogen.

The rate of urea rise may be expected to vary in different dogs depending on the condition of the bowel and on the degree of general depression incidental to experimental procedures and anesthesia. It is interesting and important that in practically every instance in which the experiment was continued to the point of apparent equilibrium, the bowel concentration of urea reached a figure somewhat above that of the blood plasma.

To obtain more information regarding the maximum bowel urea level at equilibrium we approached it from the opposite side. We started with a very high concentration of urea in the bowel. Using an 11 kgm. dog we placed 100 cc. of the normal saline solution containing 200 mgm. of

urea into the loop of small bowel. The concentration was equivalent to 95 mgm. per cent of urea nitrogen. The kidneys were left intact here as in all preceding experiments. One hour and fifteen minutes was allowed for establishing equilibrium. At this time the urea nitrogen in the intestine had fallen to 35 mgm. per cent and the blood urea nitrogen had risen to 23 mgm. per cent. At two hours and fifteen minutes the bowel had 32 mgm. per cent urea nitrogen and the blood 26 mgm. per cent, and the fluid was almost completely absorbed. Thus on reaching apparent equilibrium the bowel content became temporarily established at a point higher than that of the blood stream.

Figure 1



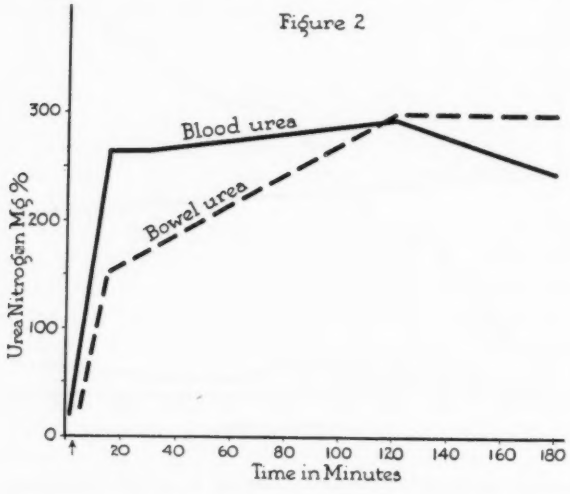
We were next concerned with the influence of a high blood urea on the equilibrium. Four dogs were used. The kidneys were removed from the body before running the tests. In each case urea was injected intravenously, allowing about 5 minutes for injection. Plain normal saline solution was used in the loop of bowel.

The results showed a rapid rise of urea in the bowel to the blood level. Figure 2 shows one experiment typical of the others. This dog received urea equivalent to 1.5 gram of urea nitrogen per kilogram of body weight. The blood urea nitrogen before injection was 16 mgm. per cent. The 15 and 30 minute specimens of blood urea were each 265 mgm. per cent. It is worthy of note that the intestinal contents reached 25 mgm. per cent in 5 minutes and 153 mgm. per cent in 15 minutes. At 2 hours the blood urea nitrogen was 290 mgm. per cent and the bowel 299. At 3 hours the blood urea had fallen to 245, while the bowel still contained 281 mgm. per cent.

One test was made on the large bowel, using normal saline in the loop, and having a normal blood urea. In 30 minutes the urea nitrogen rose to 19 mgm. per cent in the bowel. It seems that the large bowel resembles the small bowel in regard to diffusion of urea out of the blood, but our observations are not adequate to establish the equilibrium conditions.

Determinations were made on the ultrafiltrates of blood and of bowel contents. This eliminated all the protein material and gave the concentration of urea in the active fluid. The ammonia in the bowel fluid was now also determined in each case. Ammonia was not measured in the blood as a routine in the experiments, since control observations showed—and it is well known—that the ammonia content of the blood drawn under proper conditions is extremely low, too low to be a factor here. Ultra-

Figure 2



filtration showed that the actual corrected urea nitrogen of the bowel (ammonia deducted) might be as high as 17.7 mgm. per cent, while that of the ultrafiltrate of blood was 12.0 mgm. per cent. We are left with no explanation of our findings except that in some manner urea seems to be concentrated in the bowel.

With normal saline in the bowel there was always a continuous fluid absorption. Could it be that the urea passed through the bowel wall during absorption somewhat more slowly than the water in which it was dissolved? If the bowel wall is more permeable to water than to urea such a state of affairs could exist, but there would be a certain concentration of bowel urea at which urea would leave at a rate identical with that of the liquid in which it was dissolved. This then would be the point of equi-

librium. We would assume that if one started with no urea in the fluid within the bowel, urea would enter the bowel as through a partially semipermeable membrane. When a concentration equal to that in the blood stream was reached, then the continued fluid absorption would further concentrate the urea to the point of dynamic equilibrium around which it would be maintained.

If this be the proper interpretation, the inhibition of water absorption should change the equilibrium concentration of urea in the bowel to a figure exactly equal to that in the blood. We tried various fluids in the bowel to get one that would not be absorbed and finally used 5 per cent $MgSO_4 \cdot 7H_2O$. This maintained a constant fluid volume. It did not seem to cause difficulty from bowel irritation or stimulation. With the isotonic magnesium sulphate solution in the bowel the preceding experiments were in part repeated. The urea in the ultrafiltrates of the bowel content and of the blood were now identical, 12.7 mgm. per cent in the former and 12.5 mgm. per cent in the latter. The actual static equilibrium was evidently reached in this type of experiment.

Wells[1] in some unpublished work which preceded ours, and concerning which we were familiar, found that dextrose passes as rapidly into the bowel as into the blood stream if the concentrations be reversed on the two sides, indicating that the mucosa acts toward glucose as a simple semipermeable membrane.

SUMMARY

1. Urea passes from the blood into the fluid within the lumen of the bowel of dogs and vice versa, readily and in appreciable amounts.

2. With normal saline solution in the living bowel the urea nitrogen level at equilibrium has been found to be somewhat higher on the bowel side than in the blood stream. There was always rapid absorption of fluid from the bowel in these experiments.

3. It made little difference in the equilibrium finally arrived at whether one started with a high concentration of urea in the normal saline solution in the bowel or with the higher level in the blood stream.

4. When the fluid volume in the intestine was kept constant by use of an isotonic magnesium sulphate solution, then at the point of equilibrium, the bowel and the blood stream had an essentially identical urea content.

The writers wish to acknowledge the advice and assistance of Professors M.B. Visscher and A. J. Carlson during the course of this work.

[1] Wells, Herbert S., Department of Pharmacology, Vanderbilt University, Nashville, Tenn., by personal communication.

A NOTE ON THE RELATIONSHIP OF CEREBROSPINAL AND INTRALABYRINTHINE PRESSURES

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The anatomic connection between the cochlea and the subarachnoid space is a matter of common knowledge. In clinical medicine this cochlear aqueduct is one of the routes by which infection spreads from the inner ear to the meninges and results frequently in a fatal meningitis. The physiology of the relationship existing between cerebrospinal and intralabyrinthine pressures has, however, never been studied. Weed and McKibben (1919) and Weed and Hughson (1921a, b, c) investigated the relationship between cerebrospinal fluid pressure and intracranial arterial and venous pressures. In their studies they made available for the first time a method of changing cerebrospinal fluid pressure beyond normal physiological limits. This was accomplished by intravenous injections of distilled water which greatly increased intracranial pressure and also by the injection of a strongly hypertonic sodium chloride solution (30 per cent) which reduced cerebrospinal fluid pressure far below its normal limits, in some cases well below zero. At the same time careful studies of arterial and intracranial venous pressures were made and it was definitely shown that the changes in cerebrospinal fluid pressure were not dependent upon circulatory changes. The relationship, therefore, between these abnormal changes in cerebrospinal fluid pressures and circulatory pressures has been definitely established and needs no further confirmation nor discussion here. All of these observations on cerebrospinal fluid pressure were carried out on the cat.

Szasz (1922, 1925, 1926) carried out an important and extensive series of experiments on the relationship between cerebrospinal and intralabyrinthine pressures. Increase of cerebrospinal fluid pressure was produced in every instance by the use of drugs affecting arterial pressure. Reduction in cerebrospinal fluid pressure was produced by placing a cannula in the cisterna magna and allowing fluid to escape. The cochlea manometer—dogs were used exclusively—was introduced through the round window membrane. Each one of these procedures is open to criticism. No drug can change cerebrospinal fluid pressure without affecting

intracranial arterial and venous pressure which alone confirms the result of the present experiment. Repeated dissection and microscopic examination precludes the possibility of introducing a manometer needle 1.5 mm., according to this observer, without damaging some intracochlear structure. Szasz mentions the fact that the presence of blood in the capillary tube indicated damage. A much more accurate method of control is necessary and is fortunately now available. Lorenz (1932) has also investigated the effect of pressure in the labyrinth but from a standpoint which does not bear on the present discussion. Hé does, however, say that "for good hearing there must be an intact secondary tympanic membrane," an observation amply borne out in the present investigation.

Although not directly apropos to the subject under discussion a method for the study of the physiology of the ear became available approximately a year and a half ago. This method is the Wever and Bray (1930) phenomenon whereby the functional ability of the auditory apparatus can be measured. This method involves the placing of an electrode on the auditory nerve of an experimental animal and the circuit completed by a ground electrode placed in the muscles of the neck. These electrodes are led to an amplifying apparatus which in turn is connected with a telephone receiver or loud speaking apparatus and as sounds are introduced into the cat's ear they are reproduced by the receiving apparatus with absolute fidelity. Without further discussion of this method, therefore, it can be said that a method is available by which the functional ability of the ear can be absolutely controlled. Thanks to this method an accurate study of the physiology of the integral units of the ear is now at the disposal of the investigator. The effect of physiologic changes may be determined from a purely objective standpoint rather than subjective hypothesis. In all the experiments described below the Wever and Bray phenomenon has been used as the method of control.

In the course of a general investigation of the physiology of the ear a study of the effect of reduction of intralabyrinthine pressure by opening the round window membrane and permitting the escape of intralabyrinthine fluid was made and at the same time the effect produced by increasing pressure against the round window membrane was investigated. In a case of the former procedure, where it was reasonable to assume that a reduction of intralabyrinthine pressure resulted, it was found that the functional ability of the ear was decreased for the transmission of high tones. In the latter case, although it was not thought that the effect obtained was due to increased intralabyrinthine pressure (Hughson and Crowe, 1931; Crowe, Hughson and Witting, 1931; Crowe and Hughson, 1931; Hughson and Crowe, 1932) however, it immediately became apparent that careful study of the effect on sound transmission of changes in the intralabyrinthine pressure should be made. In the course of careful

and repeated experimental procedures it was shown beyond all question of doubt that no instrument could be introduced through the round window membrane and fixed sufficiently firmly in place to permit accurate pressure readings without damaging seriously intracochlear structures. With the method described above available any such procedure invariably resulted in damage to the basal osseous spiral lamina and a prompt loss in the ability of the ear to transmit high tones or if severe hemorrhage resulted, in the complete loss of transmission of all tones. Furthermore, within normal physiologic limits there was no method available for changing beyond these limits intralabyrinthine pressure unless it could be demonstrated that marked changes in cerebrospinal fluid pressure might eventually result in corresponding changes of pressure within the cochlea itself without at the same time affecting general systemic arterial and venous pressures except by the use of hypotonic and hypertonic solutions given intravenously.

Thirty experiments have been carried out on cats in the course of the past few months to determine the relationship of fluid pressures in these two body spaces. As stated above it was known that the release of fluid in the cochlea resulting from opening the round window membrane, caused a reduction in intensity of transmission of high tones by the ear. Seven experiments were carried out in which intravenous hypertonic sodium chloride solution (30 per cent) was administered and the effect upon transmission of tones through the ear measured. In every instance the result corresponded to that produced by opening the round window membrane and permitting the escape of intralabyrinthine fluid except that the effect was more striking and more marked. In two experiments hypotonic solution was given intravenously and the effect of these injections also noted. No change could be observed in the ability of the ear to transmit tones except perhaps an increased efficiency at the period of time after the injection when it was known that cerebrospinal fluid pressure had probably reached its highest level. In these early experiments careful study was made of the round window membrane after the injection of the fluid. No particular effect could be observed after the hypertonic solution had been injected because of the proximity of the basal osseous spiral lamina and the normal concave position of the secondary tympanic membrane. After injection of distilled water, however, the membrane could definitely be seen to bulge indicating an increase in intralabyrinthine pressure.

Following these observations transmission tests were made after the injection of both distilled water and hypertonic salt solution taking at the same time cerebrospinal fluid pressure readings made by means of a manometer introduced into the cisterna magna. Following the intravenous injection of distilled water or hypertonic salt solution either a sharp rise or fall of cerebrospinal fluid resulted and if the former, no change in intensity

was noted and if the latter, a marked decrease in intensity of high tones always resulted. Provided the fall in cerebrospinal fluid pressure was great enough all tones from 250 to 4000 d.v. were invariably affected (table 1). In this experiment the values of the various tone frequencies are seen to decline rapidly following the injection of 30 per cent sodium chloride solution. The transmission test made two hours after the end of the injection shows a complete loss of the high tones and a marked falling off of low tone values. No intralabyrinthine pressure readings were made in this particular experiment.

In the cat the anatomic connection between the subarachnoid space and the cochlea is relatively large. A photomicrograph of the cochlear aqueduct is shown in figure 1, its general caliber being apparent in comparison

TABLE 1

Effect of intravenous injection of 10 cc. 30 per cent NaCl solution. Experiment 175

No intralabyrinthine pressures were taken. Note the decline of intensity of all tones as C.S.F. pressure falls.

PROCEDURE	TIME	C.S.F. PRESSURE	FREQUENCY				
			250	500	1000	2000	4000
Transmission test	0 min.		30	50	50	42	63
Cistern puncture	10 min.	120					
Transmission tests	17 min.	95	30	50	47	47	73
	30 min.	95	29	46	49	47	70
Injection begun	37 min.	85					
Injection ended	47 min.	85					
Transmission tests	52 min.	56	31	47	50	90	
	1 hr. 10 min.	0	31	51	61		
	1 hr. 30 min.	−15	39	53	60		
	2 hr. 45 min.	−20	63	62	60		

to the size of the blood corpuscles which appear in its lumen. The arachnoid membrane extends almost completely through the length of the aqueduct and the loosely woven mesh work of the membrane is readily apparent. Attention is called to this fact as it will be referred to in the possible explanation of a phenomenon to be described later.

The necessity of obtaining simultaneous cerebrospinal fluid pressure and intralabyrinthine pressure readings with a study of the effect upon the two produced by intravenous injections of distilled water or a hypertonic sodium chloride solution is obvious. Careful studies were therefore made to establish some method whereby intralabyrinthine pressure changes might be determined. The standard fixed upon was that whatever technical procedure might be employed it should not, of itself, affect the ability of the ear in the experimental animal to transmit all tones in

a perfectly normal manner. Such a standard involved the introduction of some type of recording apparatus into the cochlea without damaging in any way any one of the intracochlear structures. Careful and repeated dissections of the cat's cochlea finally established that there was available one possibility and one alone for the proper estimation of intralabyrinthine pressure. Slightly to the medial side, about 1 mm. anterior from the attachment of the secondary tympanic membrane to the round window niche there is a space which permits the introduction of a needle of fine bore into the scala tympani. Through the kindness of Dr. Isidor Ravdin and Dr. Rubin Lewis of the University of Pennsylvania, a Lewis water bubble manometer was obtained. This instrument is extremely sensitive, is flexible and suited ideally to such pressure estimations. It has the further advantage that it is at all times a closed system and in so small a

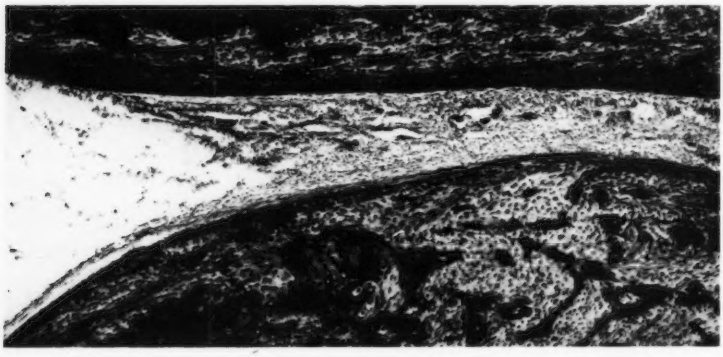

Fig. 1. Cochlea aqueduct, showing loosely woven meshwork of arachnoid membrane. In this particular section there is considerable blood in the aqueduct.

space as the cochlea obviously any open system of pressure measurement such as the open capillary manometer used by Szasz, would be far from satisfactory. Figure 2 represents the method used in introducing the needle of the manometer into the cochlea. At first a small hole is drilled in the cochlea wall using a 1 mm. dental drill burr. This hole is drilled under a binocular microscope. With sufficient experience the bone can be drilled to a point where only the thinnest shell of bone is left, figure 2 A. When the experiment is finally set up the cochlear manometer needle is forced through this final thin shell of bone, the joint made tight with ordinary bone wax and pressure readings thereafter may be recorded with the greatest accuracy. Figure 2 B shows a dissection of the medial cochlea wall, the needle in place through the drill hole and emphasizes the fact that it comes in contact with none of the cochlear structures. The

cochlear aqueduct lies almost immediately below the open end of the needle. The procedure employed in setting up the experiment is as follows:

An intratracheal tube is inserted and the animal kept under constant ether anesthesia supplied by compressed air passing over a layer of ether in a Woulfe bottle. The left mastoid bulla is then opened through the neck and the trephine prepared as described above, in the cochlea. The animal is then placed upon its abdomen, small trephines made over both lateral lobes of the cerebellum and an electrode passed through each opening so that action currents may be led off from both right and left auditory nerves. The electrodes are fixed firmly in place, the animal put upon its side, the indifferent electrode placed in the muscles of the neck and a test made for transmission of sound through both ears. This test is regarded

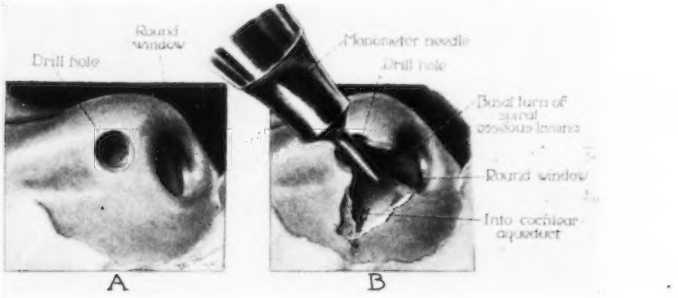

Fig. 2 A. Drill hole in cochlea wall medial and anterior to round window niche. A thin layer of bone is left until manometer needle is introduced.

B. Dissection to show position of manometer needle in cochlea.. Point of needle shown entirely free from spiral osseous lamina.

as the normal control. Following this a cistern puncture is made, the needle attached to a water manometer and after an interval of ten minutes, during which time the cerebrospinal fluid pressure usually reaches a fixed level, another sound transmission test is made, in this latter instance of course with a known cerebrospinal fluid pressure. The cochlear manometer needle is then introduced through the trephine opening, held firmly in place with a clamp and another transmission test immediately made to determine whether or not any damage has been done to the cochlear structures. If there is any striking change observed in this particular test on the side on which the cochlear needle has been introduced the experiment is abandoned and ultimate examination under the microscope invariably shows that the basal osseous spiral lamina has been damaged and that hemorrhage has occurred. If the introduction of the cochlear needle is

successful, either hypertonic or hypotonic solution is then injected intravenously in the foreleg vein. Following such an injection pressure readings are made at stated intervals and these controlled by transmission tests. As a rule readings are made every fifteen minutes for the first hour and continued thereafter at longer intervals for from two to three hours following injection of the solution. Fourteen such experiments have been performed but due to the obvious technical difficulties involved in the

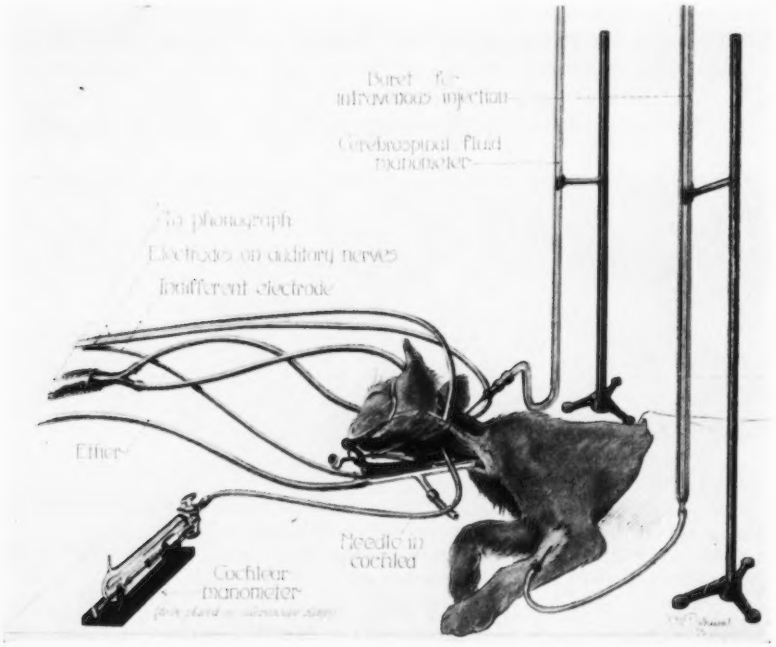

Fig. 3. General set up of experiment, showing position of electrodes, manometers, etc. The cochlea manometer is placed on microscope stage and changes in position of water bubble observed through the microscope.

setting up of the experiment it has been impossible to obtain satisfactory records in every instance. A general schematic illustration of the set up of the experiment is contained in figure 3 which is largely self-explanatory.

After finally surmounting the technical difficulties encountered it has been possible to demonstrate satisfactorily a definite relationship between changes produced in cerebrospinal fluid pressure and the consequent similar changes occurring in intralabyrinthine pressure. In the tabulations which are herewith discussed the effect produced upon sound transmission

is included. For the sake of brevity only figures pertaining to the operated ear are given. In every instance the transmission effect on the unoperated ear was in the same direction as in the operated ear. In these tabulations it will be seen that the frequencies 250, 500, 1000, 2000, 4000 d.v. have been arbitrarily chosen. Under these frequencies values in decibels of attenuation are given. These represent the number of decibels necessary to bring into balance intensity of transmission through the cat's ear and the control intensity of the dynamic microphone system. Increase in the number of decibels represents diminished intensity of transmission and decrease in the number of decibels of attenuation means, of course, improvement in transmission. A detailed description of the amplifying apparatus used and the method involved for measuring changes of intensity produced by different experimental procedures is now in print (Witting, 1932). Up to the present time no effort has been made to make a quantitative estimation of intralabyrinthine pressure changes. The Lewis manometer is approximately thirty times more sensitive than the water manometer used in recording cerebrospinal fluid pressure changes. This factor, however, has not been investigated closely and under the tabulations of intralabyrinthine pressure the direction of the change is always the same as cerebrospinal fluid pressure and for the purpose of this experiment is considered only from a qualitative standpoint.

TABLE 2

Effect of intravenous injection of 50 cc. distilled water. Experiment 169

Intensity values were unaffected by cochlear puncture and for high tones show improvement following increased pressure in labyrinth. Note change of pressures in same direction.

PROCEDURE	TIME	C.S.F. PRESSURE	INTRA-LABYRINTHINE PRESSURE	FREQUENCY				
				250	500	1000	2000	4000
Transmission test	0 min.			34	37	40	86	79
Cistern puncture	5 min.	133						
Cochlear puncture	10 min.		56.6					
Transmission tests	12 min.			30	33	44	80	80
	22 min.	147	57.1					
Injection begun	24 min.	157	58.2					
	29 min.	209	71.1					
Injection ended	44 min.	236	69.0					
Transmission tests	45 min.			30	41	40	50	60
	55 min.	250	68.1					
Transmission tests	1 hr	260	72.7	29	34	38	46	55
	1 hr. 10 min.	262	67.2	30	38	40	41	50
	2 hr. 45 min.	262		28	39	40	37	46
	3 hr.	256						

In table 2 the results of an experiment in which 50 cc. distilled water were administered intravenously are recorded. The animal was prepared as described above, the left ear used and the tabulations recorded represent as shown, procedure, time interval, cerebrospinal fluid pressure, intralabyrinthine pressure and the transmission test made at definite intervals. It can be seen that following the introduction of the cistern needle and the manometer needle for intralabyrinthine pressure no appreciable effect was produced upon normal transmission. Following injection of the distilled water cerebrospinal fluid pressure rose gradually to approximately twice

TABLE 3

Effect of intravenous injection of 50 cc. distilled water. Experiment 171

Illustrates lag in response of intralabyrinthine pressure where maximum was not reached until C.S.F. pressures had started to decline.

PROCEDURE	TIME	C.S.F. PRESSURE	INTRA-LABYRIN-THINE PRESSURE	FREQUENCY				
				250	500	1000	2000	4000
Transmission test	0 min.			27	31	40	39	56
Cistern puncture	5 min.	167						
Transmission test	7 min.			28	35	43	37	52
Cochlear puncture	15 min.	180	65.5					
Transmission test	17 min.			28	37	39	40	55
Injection begun	22 min.	180	67					
	27 min.	216	69.3					
	32 min.	235	70.5					
	35 min.	252	71.4					
Injection ended	42 min.	285	74.2					
Transmission tests	43 min.			28	47	44	70	60
	1 hr.	288	81	29	40	43	83	60
	1 hr. 5 min.	290	79.5					
	1 hr. 15 min.	258	89					
	2 hr. 45 min.	194	*	27	37	44	46	55
	3 hr. 15 min.	169		26	37	46	42	60

* Pressure in manometer rose so high that bubble was forced into bulb at end of manometer, consequently no further manometer readings could be taken.

its normal height. During this period there was a constant increase in intralabyrinthine pressure and with slight variation a general tendency toward improvement in sound transmission through the ear. The most striking fact and the one of particular interest in this discussion is that the intralabyrinthine pressure changed in the same direction and with slight variations uninterruptedly as the cerebrospinal fluid pressure increased. A final reading of the intralabyrinthine pressure in this experiment was impossible as the needle had become plugged. In table 3 an even more striking effect is apparent for all the factors involved and in addition another effect became apparent which has since been confirmed repeatedly.

It can be seen that with the increase of cerebrospinal fluid pressure there is a constant increase in intralabyrinthine pressure. However, in this experiment it was possible to make readings of the intralabyrinthine pressure over a period of two hours following the intravenous injection of distilled water and there is found to be a certain lag between the rapidity of change in the cerebrospinal fluid pressure and the intralabyrinthine pressure. In other words, after the cerebrospinal fluid pressure had reached its height and had begun to recede somewhat, intralabyrinthine pressure continued to rise. The explanation of this phenomenon seems more or less obvious. The aqueduct, although comparatively large in the cat, is still a minute structure and the interchange of fluid pressures must, of

TABLE 4

Effect of intravenous injection of 9 cc. 30 per cent NaCl solution. Experiment 176

Decline of intralabyrinthine pressure following that of C.S.F. pressure again showing lag in reaching final low level. Note marked loss of intensity of all tones with complete disappearance of two high tones following injection.

PROCEDURE	TIME	C.S.F. PRESSURE	INTRA-LABRYIN-THINE PRESSURE	FREQUENCY				
				250	500	1000	2000	4000
Transmission test	0 min.			30	40	40	60	63
Cistern puncture	10 min.	118						
Transmission test	17 min.	118		33	45	43	54	55
Cochlear puncture	30 min.		88					
Transmission test	33 min.	132	88	28	35	35	67	80
Injection begun	36 min.	130	88					
Injection ended	47 min.	130	88					
Transmission tests	50 min.	50	87	63	64	63		
	1 hr.	0	87	66	66	70		
	1 hr. 15 min.	−23	82	66	63	70		
	1 hr. 30 min.	−26	77	61	68			
	2 hr. 30 min.	5	64.3	61	68	80		
	3 hr.	20	63.2	58	53	70		

necessity, take place very slowly. In short, it is necessary for the cerebrospinal fluid pressure to reach a high level and be sustained at that point for some time before its full effect can be produced upon the intralabyrinthine space. It is also of interest in this experiment to note that this greatly increased pressure had no particular effect upon the transmission of tones through the ear, if any effect was noted, it was in the general direction of improvement of transmission. In table 4 both cerebrospinal fluid and intralabyrinthine pressures are given and the change occurring in the same direction in both pressures following injection of the hypertonic salt solution is apparent. Here again the intralabyrinthine pressure did not reach its lowest point until after the cerebrospinal fluid had itself begun to

return to its normal level. This lag in effect on the intralabyrinthine pressure corresponds exactly to that described in table 3 following the injection of distilled water. The effect of the reduction in the pressure upon transmission is also shown with a prompt loss of high tones and gradual decrease in intensity of all tones.

It is felt that the experiments described above prove conclusively the intimate relationship between cerebrospinal fluid pressure and intralabyrinthine pressure. The dependence of the latter upon the former is perfectly evident. Although the changes described in cerebrospinal fluid pressure as the result of intravenous injections of hypotonic and hypertonic solutions are obviously beyond normal physiological limits, nevertheless it would be impossible by any other method to demonstrate satisfactorily the free communication between these two cavities. The change produced upon the ability of the ear to transmit tones is equally striking though need not be gone into in great detail in the present discussion. It must be emphasized again that the present investigation has to do solely with qualitative changes in intralabyrinthine pressure. The quantitative estimation of these pressures will appear later. Furthermore, it is felt that a method has been developed whereby intralabyrinthine pressures may be obtained without damaging intracochlear structures and in which the accuracy of the pressure readings can be accepted without qualification. It is felt that no other method of determining intralabyrinthine pressure, used up to the present time can be regarded as reliable. This statement is based entirely on the fact that the introduction of the cochlear needle and the effect of its introduction can at all times be controlled by sound transmission tests, a method heretofore unavailable.

CONCLUSION

A new method for determining intralabyrinthine pressure is herewith described and the intimate relationship between cerebrospinal fluid pressure and intralabyrinthine pressure shown. When cerebrospinal fluid pressure is changed by intravenous injection of hypotonic or hypertonic solutions the rise or fall in pressure occasioned thereby is followed directly by intralabyrinthine pressure. There is a definite lag in the change produced in intralabyrinthine pressure, the extremes here not being reached until some time after the limit of change of the cerebrospinal fluid pressure has occurred.

The accuracy of this method is made practically absolute by the simultaneous use of the Wever and Bray phenomenon.

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